6. With the Authors Compliments

OBSERVATIONS
ON THE PHYSIOLOGY
OF THE
FEMALE GENITAL ORGANS,

Being a Report to the Science Committee of the
British Medical Association,

BY

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endeavoured to get some insight into the general calcium metabolism in order to control our special observations. The real work on these general questions has yet to be done by others. We trust that some enterprising physiologists, to whom the work really belongs, will take it up—if, indeed, many of the matters to which we shall allude are not already under consideration.

In any investigation of the rôle of any product of, or factor in, metabolism, it is necessary first of all to be able to isolate that body from the tissues to which its activities are chiefly confined, and to be able to estimate any changes it may undergo quantitatively and qualitatively in different circumstances.

In order to be able to investigate the variations in the blood-calcium content, one of us (W. B. B.) devised a method for the quantitative estimation of the calcium salts. A preliminary communication was made in the BRITISH MEDICAL JOURNAL, April 20th, 1907, concerning this method and the reasons for which it was devised. Briefly, it consists in precipitating the calcium in the blood in the form of crystals of calcium oxalate, and, after certain dilutions and precautions against the formation of other crystals, counting those of calcium oxalate, which are very uniform in size, on a haemocytometer plate. Any slight inequality in size is corrected by the large number counted. An average of 1 crystal per square gives an index of 1 (see Fig. 1).

Since that date the method has been investigated by other observers—many of whom have been kind enough to communicate their views and results to the author of it—and the expression of opinion has been very favourable as regards its accuracy. In this respect the author has been greatly indebted to Professor Herbert Jackson, of King's College, for the deep and friendly interest he has taken in the matter, and for the comprehensive investigations made in his laboratory into the reliability of the method. His verdict is that it is an excellent clinical method.* We are ready to admit (in the present state of chemical knowledge) that this may not be a strictly accurate chemical method, for probably only the ionized calcium is precipitated and estimated; but this is of small importance so long as the method is, as we believe it to be, *relatively* accurate. At the same time we would point out to those chemists who have criticized the method on theoretical grounds that really they have no *locus standi* until they can define and demonstrate what amount of calcium is ionized in the blood and what is not.† We may here state that the only adverse criticisms that have come to our knowledge through the medical press or otherwise have been advanced by men who have never seen the method practised! It will, however, be observed that we are meeting criticisms that have arisen or may arise, even though they may not be worth serious consideration.[1]

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* Since this paper was written, a very careful experimental criticism has also appeared. See "The Effect of the Administration of Calcium Salts and of Citric Acid on the Calcium Content and Coagulation Time of the Blood," T. Addis, *Quarterly Journal of Medicine*, January, 1909.

† In connexion with this question of "ionized" and the so-called "masked" calcium in the blood, we would point out that chemists are not all agreed. Many assert that the "masked" calcium—that is, the calcium in combination with proteids—cannot be split off and precipitated by oxalic acid. Others (see W. A. Osborne, *Journal of Physiology*, vol. xxvii, p. 400) think that oxalic acid precipitates all the calcium present.

In further reference to the method—in augmentation or qualification of the original preliminary communication—we would like to say that we have quite recognized, as, indeed, was originally indicated, that no comparison can rightly be made between a watery solution and an albuminous solution, such as blood. In fact, strictly speaking, an index should be established for every fluid for itself. We hope eventually to indicate how this may best be done so as to bring all more or less into line, for we have always recognized that albuminous or colloidal solutions are the best adapted to this method of calcium estimation. Any actual estimation mentioned in these papers will only concern blood, unless it is specifically stated to the contrary. We have taken the normal maximum in human blood to be about 1 crystal per haemocytometer square. This is an index of 1, and is based on many observations. Whether it is strictly accurate or not is of little importance at the moment, for we have only required a relative measure.

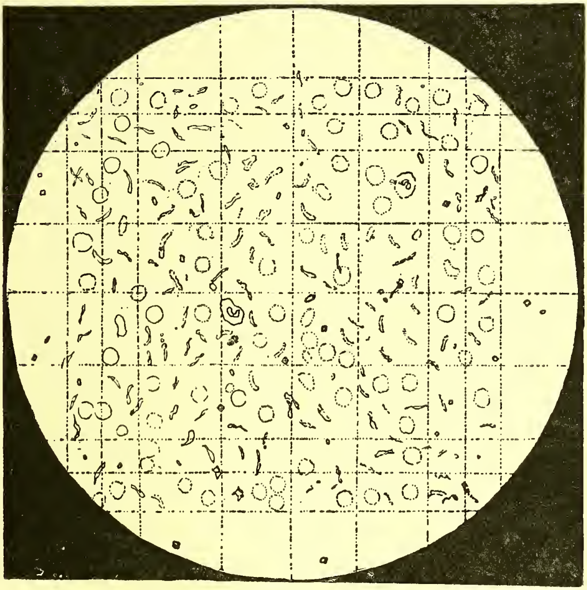

Fig. 1.— × 420. Normal blood, calcium content.

From many communications received, and from observing the work of others not conversant with the method, we think that the main difficulties have arisen:

1. In bad technique with regard to the cleanliness of the flasks, pipettes, and plates, all of which must be scrupulously dry and clean when used.
2. In the illumination and magnification. As mentioned in the original paper, only a *dull artificial* light is of any use. An 1/8 in. objective with a No. 2 eyepiece (× 420 approximately) and an Abbé condenser give the best view. We cannot overrate the importance of getting the picture correctly, otherwise it is impossible to recognize the crystals for certain.

There is one more point also alluded to in the original paper. If there is a very large number of crystals with much unevenness in size, greater dilution must be made in the *initial* stage of the process, and allowed for in the final calculation. Conversely, we may add, if there are exceedingly few or no crystals (very rare) to be seen, the *final* dilution should be lessened—say, 250 c.mm. of water should be used instead of 500 c.mm.—and allowed for in the final calculation. It has been stated that in the dilutions used calcium oxalate is soluble; we can only say that if the method is properly carried out the solubility point of calcium oxalate is never reached.

Before describing the results of the observations made we should like to discuss what value can be attributed to an estimation of the lime salts in a blood examination. In the first place, we must state that we take it as an accepted fact that the other soluble salts are concerned in the part played by the calcium salts. Secondly, we would point out that in dealing with the blood one is dealing at all times with a very variable tissue; any chemical body it contains is like an elusive Will-o'-the-wisp, which leads us a merry dance before we can come to any conclusions at all. But, after all, how can we expect otherwise? If any one ever seriously anticipated that the calcium content of the blood could be finally and definitely expressed as being one in so many thousand, as is stated in the textbooks, he must, indeed, be inflicted with laboratory vision of the worst type. These things cannot be settled by *in vitro* examination. Life, as we call it, with its never-ending changes, is a necessary adjunct to metabolism.‡

This statement, if carefully considered, should be sufficient answer to those who have written of the "curious use"[1] one of us (W. B. B.) has made of the word "index" in connexion with the calcium content. This word has passed into current medical use in connexion with many other varying metabolic factors; why not also in connexion with calcium? Far better had chemists qualify the statement to be found in textbooks that the blood contains so much calcium, estimated as CaO! It may do so in regard to the special sample that was under investigation; but there the accuracy begins and ends. The use of the word "index" makes the matter clear in regard to the method under discussion; no other word would do so.

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‡ Cf. action of "Antileukozytenserum" *in vitro* and in the circulation; it is diametrically opposite (Delezenne, *zit. nach. Jahresber. f. Tierchemie*, 1900, S. 141).

The calcium content of the blood is, then, like that of any other food supply, a variable quantity. Apparently it corresponds to some extent with the coagulation-time variations which have been worked out by Coleman[2] and others. We would emphasize the fact very strongly however—for it seems in danger of being overlooked—that the coagulation of blood depends on many factors, of which calcium is only one, and that acting within limits. We think, however, from our own observations and from a private communication we have received from T. Addis, that it will be shown that variations in the calcium content do not cause changes in the coagulation time to the extent usually believed.§

In order to assure ourselves of the diurnal variations in the calcium content we each estimated the other's blood at frequent intervals throughout one day, during which we ate practically the same food and took the same amount of exercise and rest. The accompanying table shows the result.

	W. B. B.	P. H.
Sunday, June 23rd, 1907:		
8.45 a.m.	Index, 0.7	Index, 1.2
10.0 a.m. Breakfast	—	—
11.30 a.m.	Index *	Index, 0.92
1.30 p.m. Luncheon	—	—
3.30 p.m.	Index, 0.45	Index, 0.5
3.45 to 5.15 p.m. Walking exercise	—	—
5.15 p.m.	Index, 0.65	Index, 0.56
8.0 p.m. Dinner	—	—
10.0 p.m.	Index, 0.6	Index, 0.43

* Error in technique spoilt this count; as far as could be seen index was about 1.0.

It will be seen that, with the one exception, which may not have been quite accurate, our blood contents varied regularly and together. We have not followed the quest through the night. That is a small matter of research we leave open to others—not that we would shirk the task if we thought any knowledge of use to us could be gained by performing it.

This observation, which has been confirmed by other counts, was very instructive, and useful in helping one to form conclusions as to the value of indices under varying conditions, and showed the importance of taking the blood at the same time each day when making observations on a case, or performing experiments on animals. This diurnal variation gives confirmation also to the statement we have made that the blood is the agent of the tissues in respect of the calcium salts; and an index of the blood can, therefore, be construed into an index of the condition of the tissues.

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§ Since this paper was written, T. Addis's paper has appeared. See previous footnote.

In normal physiological conditions, if the tissues are needing calcium, one would expect to find a low calcium index—that is to say, the tissues will be taking up all the calcium they can get. There are, however, other sides to the question ; for example, the calcium supply in the food may be low, or the absorptive functions in the bowel deficient ; the tissues may want calcium, and the blood be unable to supply it in the form required; or, again, calcium may find its way into the blood in a reversed direction—from breaking down tissues—as sometimes occurs in pregnancy.

We refrain from mentioning further possibilities, for such a picture appears anything but easy of interpretation. It was, moreover, the complexity of these conditions which impelled us to seek out the regulating or controlling factors in the calcium metabolism, for we felt that upon some elucidation of this difficult problem depended the correct interpretation of many blood indices, and the probable deductions to be made eventually in questions of genital activity.

Let us state at once that much remains to be done in this direction, and one of us (P. H.) hopes in the near future to follow up the full relationship of the ductless glands to the general calcium metabolism, both in its normal and pathological aspects.

That the lime salts play a very prominent part in the metabolic processes of all living tissues there can be no doubt, and it is therefore somewhat strange that until recently so little interest has been displayed in the matter. The part played in bone formation, and in the coagulation of blood and milk (comparatively recent discoveries), and the so-called "tonic" influence on cardiac muscle, founded on the splendid work of Sidney Ringer, have been the only matters to attract attention, and even these have never been followed out properly.

Lately, however, a calcium "wave" seems to have come over medical science, and admirable work has been done in different departments by Sir A. E. Wright, Sir James Barr, MacCullum, and many others; even at the present time, however, the conclusions arrived at are very imperfect, and this we venture to think is largely due to the absence of a broad and comprehensive view of the subject, which alone can correlate all the issues.

We have, therefore, kept ever before us the importance of taking a wide outlook on all the work that has been done, and have attempted to harmonize our own observations of a general nature with those of the special functions we set out to study, for those functions are not local in origin or effect, but general.

Broadly speaking, then, we have come to the conclusion that *the lime salts are not only necessary for the construction of the body and its growth, but also for keeping it healthy and in repair, and for the reproduction of the species.*

These salts only become apparently pathological—as far as we can discover—when, like that beneficent process known as inflammation, they endeavour to rescue diseased structures and to repair them. As long as the disease—arteritis, for example—continues, so long will the reparative attempts, which include a determination of calcium salts to the part, be carried on. This reparative function of the calcium salts naturally follows on the original constructive rôle. You can cause no definite lesion, medical or surgical, in an animal, nor find one in the human subject, without there being a determination of calcium salts to the injured region, as indicated, if the lesion is severe enough, by a fall of the calcium salts in the blood until convalescence sets in—that is to say, until the demand has been met or the regulators of the calcium metabolism have established an equilibrium in the altered circumstances.

This selective and retentive power of the cell for calcium salts is probably in part under the influence of the calcium-regulating factors to be discussed presently. At the same time there is every reason to believe that individual cells exhibit a self-regulating selective and retentive power in regard to all chemical compounds with which they are brought into contact.

Had we not been able to make these observations experimentally the fact has always been there for the observing. Who has not seen the calcareous deposits at the site of many an old chronically inflamed area? It is an everyday observation in the case of tuberculous disease, but has the full significance ever been realized?

We believe that there is no such thing as calcareous degeneration. What does happen is that there is a calcification—the result of a reparative process—in diseased structures.* We would refer, in this connexion, to an extremely interesting paper by O. T. Williams,[3] in which he claimed that the calcium soaps, which he demonstrated to be deposited in diseased appendices, were the cause of the condition known as appendicitis. Is it not possible, however, that these calcium compounds are there *as the result rather than the cause* of the inflammation? We would also point out that ordinary fibrous tissue, especially scar tissue, is relatively exceedingly rich in calcium salts, for the benefit of those who are at all sceptical of the deductions drawn concerning the deposition of calcium compounds.

These views, as already stated, have been entirely supported by hundreds of blood examinations, particulars of which would be superfluous here; and these observations, like those on the diurnal variations, have been of great importance in assisting us to understand the blood index.

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* The deposition of calcium soaps is an intermediate stage leading to calcification

In addition to constructive and reparative processes in which the calcium salts are retained or fixed, there are other processes no less important—indeed, it was the mystery surrounding them that led us to look for the regulating factors in the calcium economy.

Even while circulating in the blood the calcium salts play a large part in the health of the individual. We say "even while circulating," whereas, of course, we mean to speak of them in the same way as one does of the oxygen in the form of oxyhaemoglobin, or of the opsonins in the blood—in the potentialities each possesses.

It is to the metabolism associated with the rapid tissue changes rather than that associated with the retention of lime salts, already alluded to, that we now wish to call attention. Suppose that the individual is structurally and physiologically perfect for the time being, what are the processes in which the calcium salts take part? We do not pretend to know all, nor perhaps even the larger number, but we will mention briefly those which we think are most worthy of attention, and of which we have some knowledge.

In regard to these rapid tissue changes, in the first place there can be no doubt, we think, that the lime salts are largely concerned in the contraction of the involuntary muscle throughout the whole body. If we were able to abstract the lime salts from the blood, or prevent them gaining admittance, the heart would fail and involuntary movement generally would cease. Sidney Ringer's brilliant work in this direction established these facts. In a later paper, when dealing with uterine contractions, we shall illustrate the effect of calcium salts upon the heart. We do not for one moment say that involuntary muscles cannot be affected by other chemical and physical agents, but there is very strong evidence to show that the calcium salts play a large part in the production of the normal stimulus, and—to speculate—probably of the primary stimulus in the embryo. In subsequent papers we shall deal with the question of uterine contractions, and also with the processes with which calcium salts are concerned in menstruation, pregnancy, and lactation.

We have made, also, some observations in regard to the calcium content of the blood and its coagulability, and while we have not made any serious study of the question —for T. Addis, of Edinburgh, has informed one of us in a private communication that he has this matter in hand*— we would like to make a few remarks upon the subject here.

We have often been asked if there is not a certain element of danger in the free administration of calcium salts. From many observations and experiments we feel quite justified in saying No. One cannot force up the calcium index in the blood much beyond the usual maximum, in normal circumstances, either by oral administration or subcutaneous injection, and even if this were possible—to have a lawyer's alternative—clotting in the vessels, *if they were healthy*, would not occur. We have killed animals with intravenous injections of calcium salts without producing intravascular clotting, *except where the vessels had been previously damaged.* There is therefore, we think, an *optimum* amount with a quickest normal clotting time, and the latter excess of calcium salts cannot increase. Given, of course, an inflamed or damaged vessel —for example, phlebitis after typhoid fever or arteritis— and one may get a deposit of lime salts in the damaged walls (vide supra), and possibly clotting in the damaged vessel, which will be most likely to occur if the calcium *optimum* index for clotting exists.

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* See previous note.

One more point in this connexion. Sir A. E. Wright showed that the oral administration of an excess of calcium—which we presume he thought got into the blood —delayed clotting ; but apparently, from observations we have made, this is not due, as he thought, to an excess, but to a deficiency, in the blood. It is probable that after excessive dosage the body becomes immune or resistant to the absorption of calcium salts, or excessively active in their excretion, effected probably by the thyroid secretion. This point might with advantage be studied in infant feeding and rickets.

Of the many other points that have interested us in taking a general survey of the calcium metabolism we may single out two which have brought into our minds matters of some importance.

If we are right in our main belief that the calcium metabolism is largely concerned in the physiological processes of women, may not the alteration in the calcium economy at the menopause be to some extent accountable for the deposition of fat? This does not of course coincide with the general ideas concerning obesity and the allied metabolic processes to which that condition is usually attributed; but in view of the close connexion between certain fatty acids and calcium metabolism we feel that there may be some association which future investigation will lay bare. Excellent work on the relationship of these fatty acids to one another in regard to the absorption and deposition of fats, and on the formation of calcium soaps has already been carried out by O. T. Williams (loc. cit.).

The other suggestion we would make is this: May not the deficient excretion of calcium soaps by the bowel in physiologically active women be the cause of chronic constipation—an ever-present evil with the female sex? We think these points are well worthy of bio-chemical investigation.

Before discussing the regulating factors it may be well to include here an observation which has given rise to some controversy. While looking for a suitable organic compound of calcium for hypodermic injection it occurred to us to try gelatine, which, as is well known, contains a large amount of calcium; indeed, it has been suggested that the power of gelatine, when used therapeutically in cases of aneurysm to influence the coagulability of blood, is due to the contained calcium.[4]

We were consequently led to investigate this animal product, and found that by the method already described it was possible to estimate the calcium. Though, of course, not strictly comparable with blood, we may state that, although the commercial samples vary, we found the average index to be about 20. It will be readily understood how this quantity might influence the blood of the individual into whom it was injected. As yet no direct experiments have been done in this direction, but we found that intravenous injection of 4 per cent. gelatine solution did not cause intravascular clotting. Gelatine corresponds, therefore, with other calcium compounds in this respect, and by this behaviour tends to confirm the suggestions that its calcium content is responsible for the raised coagulability, if this does occur, rather than any nucleo-proteid-like substance.

The following protocol illustrates the action of a 2 per cent. solution of gelatine subcutaneously injected upon the systemic blood calcium content.

	White Buck Rabbit.	
November 1st, 1907 :		
9.45 a.m.	Index 0.67	10 c.cm. 2 per cent. gelatine solution subcutaneously injected.
11.45 a.m.	,, 0.67	
12.45 p.m.	,, 0.7	
3.45 p.m.	,, 0.7	
5.40 p.m.	—	10 c.cm. 2 per cent. gelatine solution subcutaneously injected.
5.55 p.m.	Index 0.76	

If, therefore, the gelatine does raise the calcium content of the blood a 2 per cent. solution does not appear to be strong enough. It is only fair to state that Boggs[5] found the period of increased coagulability to be at a maximum as long as twelve hours after the injection. The diverse results obtained in the experiments of Boggs and the conflicting opinions of Dastre and Floresco[6] on the one hand, and those of Brat[7] and Sackur[8] on the other, regarding the influence of gelatine upon the coagulability of the blood may be explained on the theory of immunization we have already alluded to, or—and this is more likely—on the ground that the methods employed by them for estimating the coagulability were inaccurate.

It was in connexion with the coagulation, or "setting," of gelatine that we made some observations which have given rise to controversy. The results of our experiments led us to ask if it was not possible that the presence of calcium was necessary for the occurrence of this process. In October and November, 1907, we carried out a series of experiments in which we tried the effect upon the coagulation of gelatine of solutions of the following substances: oxalic acid, acetic acid, ammonium oxalate and sodium citrate.† We do not think it necessary to give the protocols in detail, but will briefly summarize our results.

We found that we obtained a heavy white precipitate of calcium oxalate with ammonium oxalate and oxalic acid, and that the salts above mentioned caused marked retardation of the coagulation time. Further, that the acids (if the gelatine was in weak solution) also retarded the coagulation under ordinary circumstances; but that if the solutions were kept at a temperature which would prevent gelatinization for about forty-eight hours, that process did not occur at all. Further, we found that the *prolonged action of ammonium oxalate, but not of other salts, prevented coagulation.*

Now, in regard to the criticisms raised with reference to the suggestion that it might be worth while for chemists to discover if calcium was really an integral part of gelatine, and whether gelatine existed as such without calcium—in the first place, let us state that in all the papers quoted to disprove our suggestion (it has never been more than a suggestion) there is no mention of calcium-free gelatine having been obtained. Morner[9] and many others have done their best to obtain calcium-free gelatine, but as far as we know no one has succeeded. It seems to us, therefore, that, whether our suggestion is right or wrong, it is hardly logical to argue that because a certain percentage has been removed without interfering with the coagulability, calcium has therefore nothing to do with the process. In regard to this point, Addis's work on blood coagulation is of much interest.

Nor does it appear reasonable to assume, as has been done,[1] that because in certain experiments *delayed* coagulation only was obtained, therefore the calcium cannot be concerned. We have secured prevention of coagulation, but to that we do not wish to attach undue importance, although such positive evidence is certainly of more value than the negative evidence just alluded to.

It has also been suggested that the hydrogen ions in the oxalic acid are responsible for the result. This seems to be quite as indefinite a statement, and one which requires some proof. However, until calcium-free gelatine has been obtained which will gelatinize, and so prove our suggestion to be wrong, we would commend to unbiassed observers the following points which seem to us to be worth consideration in any attempt to settle the question:

If calcium is a factor in the coagulation of blood—and we believe that this is usually accepted—and oxalic acid and ammonium oxalate prevent that coagulation by precipitating the calcium, is it not possible that the same result obtained in the case of gelatine is due to the same cause? Again, most salts retard the coagulation of blood just as the "setting" of gelatine is delayed by them. C. J. Martin[10] attributes the result obtained by citric acid and citrates in the case of the blood to an alteration in the ionization of calcium; and this is, we believe, universally accepted. Is it not possible to apply the same idea to the action of these and similar compounds upon gelatine? This is a small matter, perhaps, and we have no wish to discuss it at greater length, as it in no way concerns our work; but, having made a promise to deal with any criticisms raised,[1] we have therefore attempted to fulfil it.

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† Compare these experiments with the statements made by H. E. Roaf (Reference [1]).

In our investigations we have so far touched but slightly upon the importance of the ductless glands in the regulation of the calcium metabolism. Time has not allowed that complete inquiry which will, as already mentioned, be pursued later by one of us. There are several points, however, to which we desire to draw attention, for they seem to have some relation to genital activity.

First, in regard to the thyroid gland. Clinically, we had observed that in most cases of exophthalmic goître, where the thyroid secretion was in excess, the blood calcium index was low, an observation which caused us to suggest to Sir James Barr that calcium salts might possibly have some therapeutic, or at least symptomatic, advantage in this disease. Sir James Barr very kindly gave these salts a trial, with undoubted advantage in many cases, the pulse-rate being markedly lowered.

Following this up, we excised the thyroid, and as far as possible the parathyroid glands of four cats. Two were quite young, and two full grown. Two died in a few days—one young and one adult—the former with tetanic convulsions on the fourth day, and the adult a few days later with slight spasms and great muscular weakness.

The other young cat died at the end of fourteen days, much emaciated, and with great muscular weakness. The last—a large black tom-cat—was becoming lethargic and myxoedematous, so we castrated him. From that time he steadily improved, and is at present quite lively and particularly intelligent. His coat is perhaps a trifle dull and dusty-looking, otherwise there is nothing at all indicative of myxoedema.* This effect of castration, if one can claim so much in a solitary instance, requires further investigation. We have been told that Stanley Kent obtained the same result, but never published an account of his experiments.

With regard to the observations made on the blood of these cats, in each of the *young* cats the calcium index ran up to a remarkable extent, while the older cats were not affected in the same way. All the animals lost weight.

The following are the protocols of these experiments:

I. *Large Tortoise-shell Tom-cat.*

November 25th, 1907. Index 0.78. Weight 3,350 grams.
Complete thyroidectomy.

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* Recently an improvement in the condition of his coat has been brought about by the administration of thyroid gland extract.

November 28th, 1907. Not well; lying very quiet. Slight muscular twitchings.

November 29th, 1907. Very quiet. Spasms when touched. Some muscular weakness. Index 0.74. Given 5 c.cm. thyracol solution and 5 c.cm. 20 per cent. gelatine solution.

November 30th, 1907. Better. Still weak. No spasms. Index 1.3.

December 1st, 1907. Died during night. Weight 2,800 grams.

II. *Young Tortoise-shell She-cat.*

November 25th, 1907. Index 0.48. Weight 1,550 grams. Complete thyroidectomy.

November 28th, 1907. Very restless; quite strong.

November 29th, 1907. In continual tetanic convulsions; cannot stand. Index 5.5. 5 c.cm. thyracol solution hypodermically and 5 c.cm. gelatine solution 20 per cent.

November 30th, 1907. Convulsions more intermittent. Still violent. Index 9.0. 10 c.cm. thyracol solution given hypodermically.

December 1st, 1907. Better. One slight convulsion. Very weak. Index 3.0.

December 2nd, 1907. Died during the night. Weight 1,240 grams.

III. *Young Black Tom-cat.*

November 27th, 1907. Index 0.67. Weight 1,750 grams. Complete thyroidectomy.

November 29th, 1907. Apparently quite well.

December 1st, 1907. Index 2.7.

December 5th, 1907. Index 14.0. Cat getting weak.

December 6th, 1907. Index 15.0. Cat much weaker.

December 7th, 1907. Index 11.0. Cat still very weak, but seems a little brighter.

December 8th, 1907. Index 12.0. Cannot use hind legs. Injection of 5 c.cm. thyracol.

December 9th, 1907. About the same condition. Weight 1,210 grams.

December 10th, 1907. Index 7.0. Cat seems much better. Injection of 5 c.cm. thyracol.

December 11th, 1907. Index 18.0. Cat seems not nearly so well to-day.

December 12th, 1907. Died during the night.

IV. *Large Black Tom-cat.*

November 27th, 1907. Index 0.3. Weight 3,250 grams. Complete thyroidectomy.

November 29th, 1907. Apparently well.

December 1st, 1907. Index 0.7. Apparently well.

December 5th, 1907. Index 0.5. Very quiet.

December 6th, 1907. Index 0.4. Rather weak.

December 7th, 1907. Index 0.8. Seems better than he was yesterday.

December 8th, 1907. Index 0.4. Seems strong but very lethargic.

December 9th, 1907. Index 0.4. So dull and apathetic that he is apparently developing myxoedema. Weight 2,890 grams. Castrated.

December 10th, 1907. Index 0.8.

December 11th, 1907. Index 0.6.

December 12th, 1907. Index 1.0. Quite active and bright. Stole some fish.

December 23rd, 1907. Index 1.0. Apparently normal.

We likewise operated upon three rabbits, but in none of these did we get the results obtained with cats. Beyond some temporary loss of weight they showed hardly any symptoms at all; nor did the calcium indices show marked increases. It will be noted, however, that the only one at all ill was the *young* one. The primary drop in the calcium content which follows all operations occurred, and was followed by a gradual restitution to the normal. This is seen from the following protocols:

I. *Grey and White Buck.*
November 23rd, 1907. Index 0.8. Weight 2,700 grams.
November 24th, 1907. Complete thyroidectomy.
November 25th, 1907. Index 0.57.
November 26th, 1907. Index 0.45.
November 27th, 1907. Index 0.3.
November 28th, 1907. Index 0.4. Weight 2,550 grams.
November 29th, 1907. Index 0.7.
November 30th, 1907. Index 1.0.
December 2nd, 1907. Index 1.1. Weight 2,410 grams.
December 4th, 1907. Index 0.72. Weight 2,340 grams.
December 11th, 1907. Index 1.0. Weight 2,420 grams.
December 18th, 1907. Index 0.8. Weight 2,640 grams.

II. *Brown Buck.*
November 23rd, 1907. Index 0.8. Weight 2,140 grams.
November 24th, 1907. Complete thyroidectomy.
November 25th, 1907. Index 0.54.
November 26th, 1907. Index 0.57.
November 27th, 1907. Index 1.2.
November 28th, 1907. Index 1.0. Weight 2,140 grams.
November 29th, 1907. Index 1.1.
December 9th, 1907. Index 0.62. Weight 2,120 grams.
December 16th, 1907. Index 1.7. Weight 2,260 grams.
December 20th, 1907. Animal seems ill.
December 21st, 1907. Died. *Post-mortem* examination showed intestinal obstruction. Intestines much matted together and distended. This was evidently the cause of death.

III. *Black and White Doe (not full grown).*
November 23rd, 1907. Index 2.0. Weight 1,510 grams.
November 24th, 1907. Complete thyroidectomy.
November 25th, 1907. Index 1.4.
November 26th, 1907. Index 0.7.
November 27th, 1907. Index 0.4.
November 28th, 1907. Index 0.2. Weight 1,400 grams.
November 29th, 1907. Index 0.56. Rabbit seems ill. ½ c.cm. thyracol given hypodermically.
November 30th, 1907. Index 0.74. ½ c.cm. thyracol given hypodermically.
December 2nd, 1907. Index 1.2. Weight 1,410 grams. Rabbit better.
December 4th, 1907. Index 0.9.
December 7th, 1907. Index 0.9. Weight 1,320 grams.
December 11th, 1907. Index 1.8. Weight 1,310 grams.
November 18th, 1907. Index 0.87. Weight 1,340 grams.

It is, however, well known that rabbits and herbivorous animals generally do not exhibit the effects of complete thyroidectomy and parathyroidectomy (if the latter is effected, which is somewhat doubtful) to the same extent as carnivorous animals. This probably explains the results of our experiments on rabbits.

There can be little doubt, we think, that the thyroid secretion plays some part in the calcium metabolism along, perhaps, with other functions, and the evidence all seems to point to an excretory effect.

Scordo and Franchini (Grocco's Clinic)[11] have recently arrived at the same conclusion from a careful examination of the calcium excretion in the urine of patients suffering from exophthalmic goître. On the other hand, MacCallum and Voegtlin,[12] in their work on the parathyroids and calcium metabolism, arrive at a different conclusion. There must be, however, some connexion between the secretion of the thyroid gland and the calcium metabolism, even if only an intermediary one, and we shall allude to this in a later paper in regard to thyroid activity during menstruation and pregnancy.

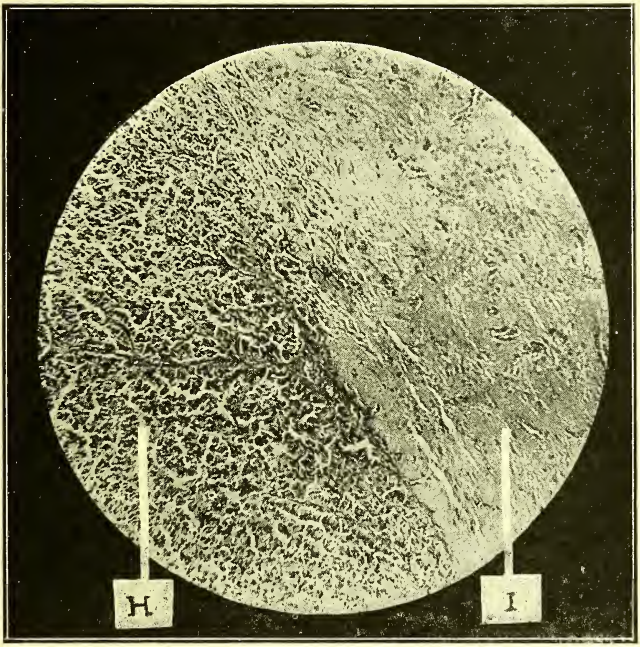

Fig. 2.—Pituitary gland (×250). Note sharp line of demarcation between hypophysis and infundibulum.

Next, the pituitary gland has engaged our attention. It is hardly necessary to say that the gland has frequently been found to be diseased in connexion with bony overgrowths, such as one finds in acromegaly; consequently it is natural to imagine that the secretion of the gland is normally concerned in the limitation of bony deposition, or calcium retention. But, further, it is found that the extract made from the infundibular portion of the gland (Fig. 2, I)—strangely enough the non-glandular portion—has an enormously powerful influence upon involuntary muscle fibre. This effect is most marked upon the uterus, as we hope to show in a later paper. The glandular portion of the gland (Fig. 2, H) is inactive in this respect. Mr. H. H. Dale, of the Wellcome Laboratories, who has kindly furnished us with the extract, has done much good work on this subject. Professor Schäfer, however, was the first to remove the pituitary gland from the lumber room of "embryonic relics."

We have reason to believe that further investigation will show that an intimate connexion exists between the pituitary extract and the calcium metabolism. Our experiments have been limited in number, but seem to show that under the influence of pituitary extract there is an increase of calcium in the blood, whether it is a normal or permanent effect we are not at the present moment prepared to say.

Again, adrenal extract, as every one knows, has not only a marked influence on involuntary muscle, but also causes calcium retention in the tissues. Acting upon this knowlege, Bossi[13] of Genoa has found that osteomalacia can be much improved by the administration of this product, and experimentally it has been shown that calcareous retention in the blood vessels is brought about by its influence.[14] This, however, may be a natural process consequent upon an arteritis.

Lastly, the ovaries (and testes possibly also) influence calcium metabolism, as is shown in many ways: osteomalacia has been cured by oöphorectomy, and we know that calcium retention occurs after the menopause. It is possible, also, that the part played by the internal secretion of the ovary in menstruation and pregnancy has reference to the calcium metabolism concerned in these processes.

This syndrome of facts leads one to many speculations—speculations which must be checked by careful experimentation. It seems, however, more than probable that the ductless glands preserve a balance in the calcium metabolism—as they do in many other metabolic processes—one acting anabolically, another katabolically.

It is sufficient for our present purpose to know that there is some important relationship, but we hope in the subsequent papers to indicate and illustrate more fully the connexion that exists between these glands and the functions of the female genital apparatus.

REFERENCES.

[1] See Correspondence, BRITISH MEDICAL JOURNAL, March 14th, and 21st and April 4th, 1908. [2] *The Biochemical Journal*, vol. ii, No. 4, p. 387, 1907 [The methods used by Coleman, Wright, Brodie and Russell, and others, have been seriously questioned.—W. B. B., P. H.]. [3] O. T. Williams, The Etiology of Appendicitis, *Liverpool Med.-Chir. Journal*, July, 1908. [4] Zibel, *Münch. med. Woch.*, 1901, S. 1643; Gley and Richard, *Comptes Rendus Soc. de Biol.*, 1903, Bd. 55, S. 464. [5] T. Boggs, *Deut. Archiv f. klin. Med*, 1904, 79, xxix, S. 539 [The methods used by him have also been questioned.—W. B. B., P. H.]. [6] Dastre and Floresco, *Archiv. de Physiol.*, 1896, S. 402. [7] Brat, *Berl. klin. Woch.*, 1902, S. 1146 and 1170. [8] Sackur, *Grenzgebiete*, 8, S. 188. [9] Morner, *Zeit. f. Physiol. Chem.*, 1899, Bd. xxviii, p. 471. [10] C. J. Martin, A Communication to the Physiol. Soc., Lister Institute, 1904. [11] Scordo and Franchini, *Il Policlinico*, 1907, xvi, p. 285. [12] MacCullum and Voegtlin, *Johns Hopkins Bulletin*, March, 1908. [13] Bossi, *Zentralbl. f. Gynäk.*, Nos. 3 and 6, 1907. [14] Wells, *Chemical Pathology*, p. 545, various references

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II.—MENSTRUATION.*

Our work on menstruation has for the most part been taken up with investigations into the relationship existing between this function and the calcium metabolism, and we have used in our deductions facts and observations which we demonstrated in connexion with the general calcium metabolism—alluded to in Paper I—as well as those which we now present as the result of our experimental and histological researches into the physiology of the function itself.

As one of us (W. B. B.) has already stated, the idea of some such relationship existing was engendered by morphological considerations in regard to certain egg-laying species, especially birds, which excrete lime salts in large quantities during this process from the calcium chamber, or lower part of the oviduct.

It has been asked why we used birds in these experiments. The answer seems so obvious that we are afraid we may have mistaken the point of the inquiry. Hens were used because we presume that no one will deny that they do excrete calcium in this way, and we thought that if we could prove certain histological and experimental analogies between the oviducts of hens when laying and human and other mammalian uteri when menstruating, we should have done all that could be reasonably required to prove the hypothesis we had set forth—apart from further evidence, of a more clinical nature, we are prepared to offer.†

First of all, then, we examined the systemic blood of three hens on consecutive days for about a week. We stipulated that one at least should be a non-layer for a control, but otherwise we were unaware of the eggs laid until the experiment was completed, although, of course, our charts soon gave us an inkling of what was happening.

From the charts which illustrate the results obtained it will be seen that the first, or control hen, showed practically no changes in the calcium index from day to day. The second hen, laying every other day, showed a marked drop after laying an egg, with a big rise the next day in preparation for the laying of an egg on the alternate day. The third hen was very interesting. An egg was laid on the first day and the index was low. This index remained low for several days, consequently no egg was laid, but finally the index rose and remained so high that an egg was laid on every day during the rest of the observation. The calcium content may have dropped for a short time after the laying of the egg, but the fall was of such short duration that it was not observed—a rapid rise, or maintained high index, being necessary for daily egg laying.

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* Based upon a paper read by W. Blair Bell before the Gynaecological Section of the Royal Society of Medicine, July 9th, 1908, "Menstruation and its Relationship to the Calcium Metabolism."

† We may call attention also to the fact that the lowest known mammal (*Monotreme*), the ornithorhynchus, lays calcium-coated eggs. If any one can furnish us with a female specimen of this rare creature we shall be extremely pleased.

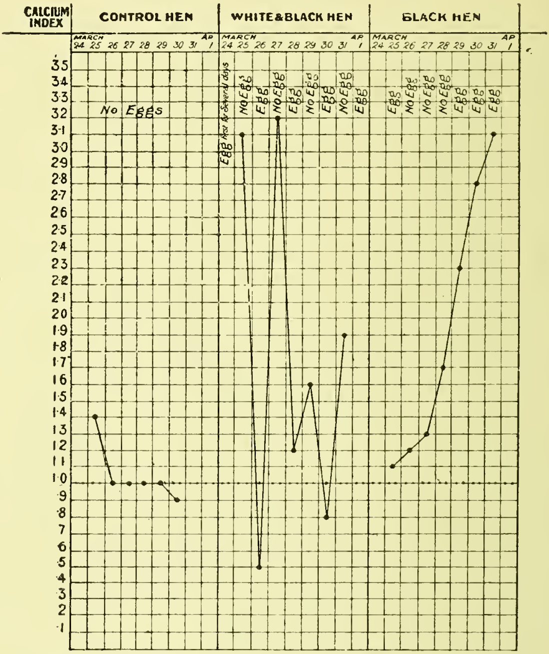

Fig. 1.—Systemic blood of hens—calcium indices.

We should like to hazard the suggestion here that the well-known vaso-dilatation which occurs in the combs and wattles of laying hens is due to the drop in the calcium content of the blood whereby a sort of chilblain condition is produced.

Now these experiments are very important, and we think that the result was only what one would be reasonably led to expect if the matter were considered at all.

It has been stated by Dr. Amand Routh when discussing the paper of one of us,[1] that he "could not see the force of the charts showing the variations of the lime in the blood of the hen which laid eggs on alternate days, for the lime for any particular egg was required not on the day before it was laid, but several days before; and many eggs were in the process of development all the time." Now we cannot see where an explanation is required. Surely a comparison between Chart 2 and Chart 3 (Fig. 1) makes matters quite clear. If the hen is to lay *every* day, it must maintain a high index, with at most a drop for a few hours. If it cannot do this, it will only lay when it has the requisite high blood content which in Chart 2 occurred on alternate days. In these experiments the eggs were, as is usual, laid in the morning, and the blood estimated in the afternoon. Further, in reference to the statement that many eggs are in the process of development at the same time, we would point out that this is only true of the ovum and yolk sac, and that only one egg receives the further coverings of albumin and shell at one time, *these processes being completed in a few hours.*

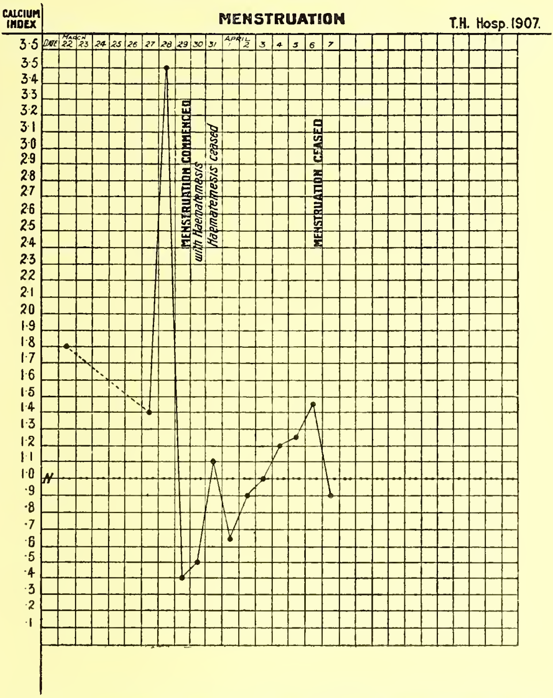

Fig. 2.—Systemic blood calcium indices during vicarious menstruation.

We will now deal with some facts and experiments which are of themselves just as convincing in regard to animals and the human subject. Having got the idea in regard to the existence of some connexion between the calcium metabolism and the female genital organs it of course at once struck us how easy it was to explain on this theory the fact that menstruation is nearly always in abeyance during lactation, for we know what an important and physiologically predominant ingredient of milk calcium is. It was easy to understand, too, why menstruation should frequently be absent in the debilitated, since we had already observed the important part the calcium salts play in the repair of diseased structures,[2] and that this form of amenorrhoea is invariably cured by the administration of lime salts; and why this function only commences when the economy has lime salts to spare from the constructive claims of the body.

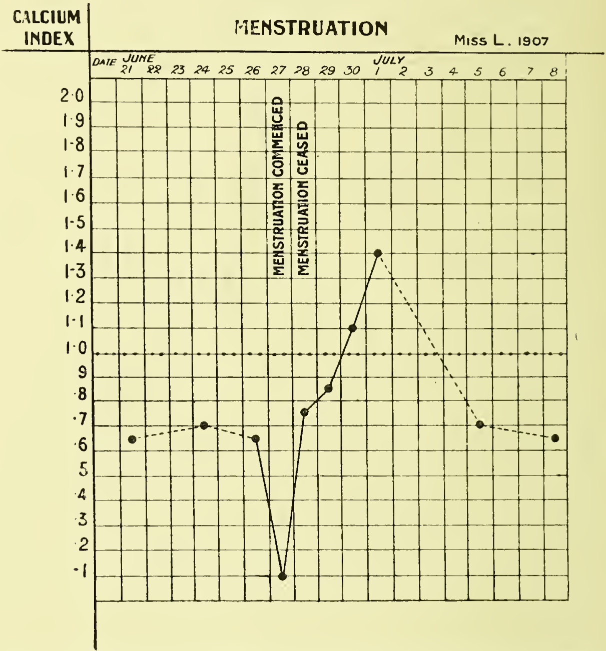

Fig. 3.—Systemic blood calcium indices during menstruation, with hystero-epilepsy.

To come to the actual observations on women. First, in regard to the systemic blood. There is always a very marked drop in the calcium content just before the menstrual bleeding commences. Often this drop is preceded by a marked rise (compare Reference [3]). The accompanying chart (Fig. 2) illustrates both of these points. It is the chart of a girl who is subject to haematemesis each menstrual period—a true case of coincident vicarious haemorrhage. We had her in hospital some time under observation.

The next chart (Fig. 3) illustrates the systemic blood content in another class of case. The patient was suffering from minor hystero-epilepsy, due to calcium deficiency, and was completely cured by the judicious administration of calcium lactate. Her premenstrual index was low. With the onset of bleeding, which only lasted for twenty-four hours, there was a marked drop. Though not so apparent as in the last case, it is almost as great relatively, in that the content dropped to one-sixth of the previous amount.

It is not easy to obtain the chart of an absolutely normal menstrual cycle We hardly remember seeing a woman who was without a single symptom at this period; but the third chart (Fig. 4) illustrates what we consider to be as normal a chart as it is possible to obtain from the human subject. In this one sees a slight rise followed by a drop—in some cases one sees two or even three abortive attempts, as it were—a further rise follows, then a big drop occurs with the onset of menstruation and any attendant symptoms the woman may periodically suffer from. In this case the patient suffered from pain and vomiting. As has been already pointed out elsewhere, it is important that the blood should be taken for the estimation of calcium at the same time each day.[2]

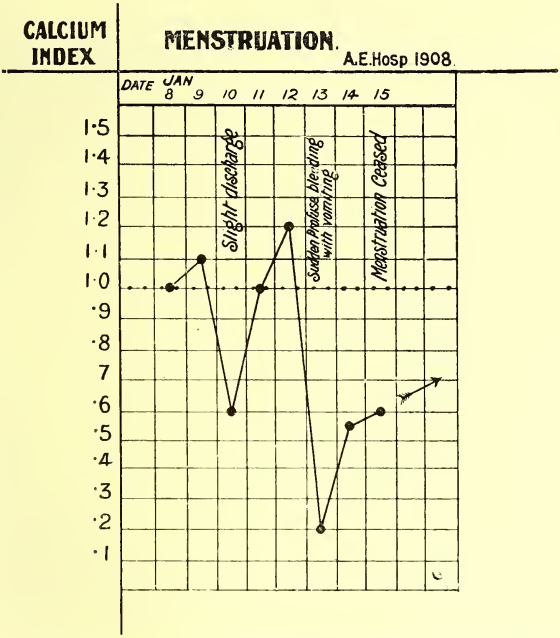

Fig. 4.—Systemic blood calcium indices during normal menstruation.

There is, then, a marked drop in the systemic blood calcium content of the human subject, which is mos marked just before the bleeding occurs. In the same way, the formation of an egg leads to a depletion in the systemic blood of birds; and in considering this we were next led to examine the excretion of the uterus during menstruation.

At the very commencement of menstruation the discharge is largely composed of leucocytes, and this local leucocytosis, which usually precedes the actual bleeding, is extremely interesting. For an examination of the discharge at this period by the method for calcium estimation mentioned shows that there are not only a large number of free crystals, but also a far larger number precipitated within the white corpuscles themselves (Fig. 5). This was so surprising to us that we should have hesitated to give credence to what we saw, or thought we saw, or to have drawn deductions therefrom, had not other more authoritative observers than ourselves kindly examined the specimens and agreed with us. Professor J. E. S. Moore informs us that in some of the lower forms of life excretion is carried out by means of leucocytes which may come to the surface and be thrown out, or even go back after discharging their contents.

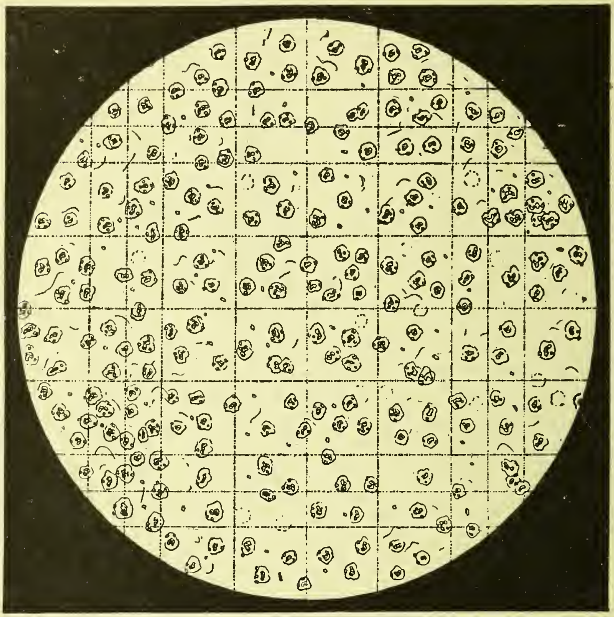

Fig. 5.—(×420.) Menstrual discharge, first day; leucocytosis.

Now this is probably an instance of what has been called pinocytosis.[4] We have to thank Dr. R. J. M. Buchanan for calling our attention to this subject, which he did after one of us had stated elsewhere[2] that it was probable that the leucocytes absorbed the calcium in a fluid form, and carried it from the glands to the exterior, possibly preventing a free secretion of calcium compounds which might increase the coagulability of the blood *in utero*. We shall allude to this point again in a moment.

An examination for calcium salts in the menstrual discharge at a later period—third to fourth day—shows that there is still a marked excess as compared with the systemic blood; but the discharge is then sanguineous and contains the usual vaginal epithelial cells with but a few leucocytes, and it shows a marked tendency to clot (Fig. 6). Thus it will be seen that there is an excretion of calcium coincident with the fall in the blood of the calcium content.

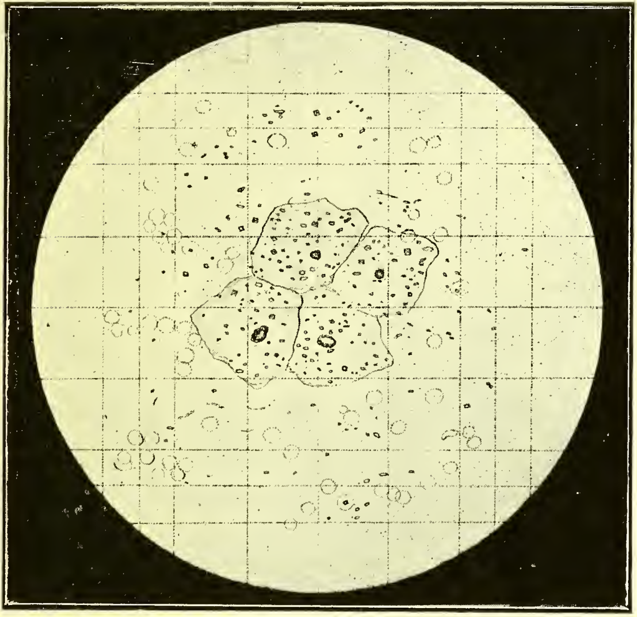

Fig. 6.—(×420.) Menstrual discharge, third day.

Further, to prove these views we tied the uterine horns of rabbits and examined the contents. The fluid thus collected was found to be exceedingly rich in calcium—more so in the adult menstruating rabbit than in the virgin. In Fig. 7 is seen the fluid collected from a young non-menstruating virgin rabbit. Here one finds the calcium combined or mixed with mucin with which it is precipitated. In the older menstruating rabbit it exists in larger quantity, free, as well as with the mucin and leucocytes, as is seen in Fig. 8.

We may here mention that the secretion from the Fallopian tubes contains very little calcium in comparison with the quantity secreted by the uterus. We performed several experiments to test this point.

To complete the chain of evidence the menstruating uteri of women and rabbits were next examined histologically.

We found, as others have done, that there is a marked activity in the glands at this time, with a diapedesis of leucocytes in the neighbourhood. In Fig. 9 is seen a section of the menstruating rabbit's uterus under a low power. In Fig. 10 a gland from the same section is shown under a high power. It is a gland in a state of activity. Leucocytes are seen outside the gland, and in the process of making their way between the gland epithelium. To this we would call special attention. It will be noticed that they become elongated and flattened. They were recently observed and figured by Marshall and Jolly[5] in this condition, but these observers concluded that these elongated cells were merely gland epithelial cells in an actively secreting condition. Ernst Holzbach[6] also mentions the same cells, and comes to the same conclusion as Marshall and Jolly.

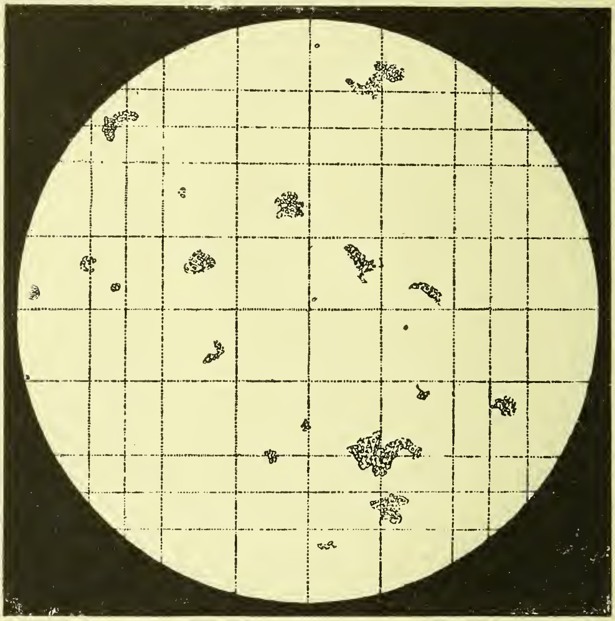

Fig. 7.—(×420.) Fluid from artificial hydrometra of young virgin rabbit.

Lastly, it will be observed that there is a collection of leucocytes within the gland lumen, embedded in the secretion of the gland. We believe that these leucocytes then carry some calcium compound with them to the exterior. Apparently the columnar epithelium that lines the interior of the uterus plays the same part as that lining the glands, which are formed by a process of invagination from the surface.

In Fig. 11 the same thing is seen happening in a gland from a menstruating woman's uterus. We have not space here to discuss the other points of histological interest, for they are many and somewhat controversial. But we cannot pass on without illustrating the histologica analogies to be observed in the calcium chamber of the hen. In Fig. 12 is seen a section (low power) of the calcium-secreting chamber of a non-laying ("sitting") hen. In marked contrast with this is a section under the same power—shown in Fig. 13—of the calcium-secreting chamber of a laying hen. In the former the *rugae* or papillae are small, and the stroma underlying the epithelial layer full of cells which have hardly any definite glandular formation (as is seen in Fig. 14). In the latter (Fig. 13)—from the laying hen—the *rugae* are large and there is a great regularity of actively-secreting glands, as will be seen in the high-power sections (Figs. 15, 16, and 17).

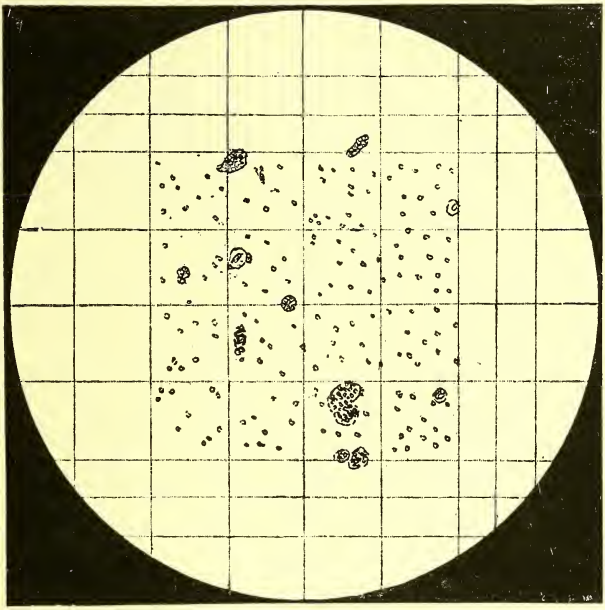
Fig. 8.—(×420.) Fluid from hydrometra of menstruating rabbit.

In Fig. 15 there are two points of interest to which we attach some comparative importance. At one spot is seen a rupture in the continuity of the epithelial surface, such as can be seen in one place in the menstruating rabbit's uterus (Fig. 9), and directly opposite to this is seen the secretion collected and infiltrated with leucocytes outside the mouth of a gland, a condition frequently seen in the menstruating uteri of women and rabbits, and, we suppose, in other mammals.

Fig. 16 shows an invagination forming a superficial gland in the calcium chamber through the epithelial cells of which leucocytes may be seen passing. Fig. 17 illustrates the vascularity and diapedesis which is seen in these *rugae* when actively engaged. Figs. 15 and 17 were taken from a hen while an egg was in the process of being coated with lime salts.

Since it has recently been established by Heape[7] and others that the pro-oestrum stage of the "rut" in animals *is* really analogous to the human menstruation, these facts are of considerable importance, and we think sufficiently demonstrate the cause and effect we wish to emphasize.

There are, however, a few other points which must be considered in order to get on to *terra firma*, and into a region from which we can glean facts of importance from a clinical point of view. In the first place, let us briefly consider the periodicity of menstruation and its significance. From the very earliest times this periodicity has been the subject of much mystery, more speculation, and not a little unconscious humour. As a result the periodicity has been allowed to form the chief stumbling-block to a solution of the problem which has been so long *sub judice.*

And yet after all there is no mystery, nor is there anything supernatural or obscure about what regularity there may be in the appearance of the catamenia. We have ceased to wonder at the rhythm of respiration and cardiac contraction—we now know that the basis of all the causative factors is founded upon chemical changes—anabolic and katabolic in nature—and that these changes themselves are entirely dependent for their regularity upon the general metabolism in whole or in part. However little understood or insufficiently explained there is little doubt that other factors are merely correlated.

The periodicity of the menstrual function—of the "rut" and breeding in animals—is exactly on the same lines. Merely a question of whether or not certain necessary articles—among them calcium—are at the disposal of the organs concerned for excretion at certain times. To some extent Stevenson[8] argued on the same lines in discussing his "menstrual wave" theory in very general terms. This periodicity has really no immutable law of a fixed time, as one might be led to think by the way some authorities have written of it. It is subject to many influences, for the individual has first claim upon all ingredients necessary for herself before she has any to spare for excretion—that is, for supplying a fresh individual (her embryo) or for

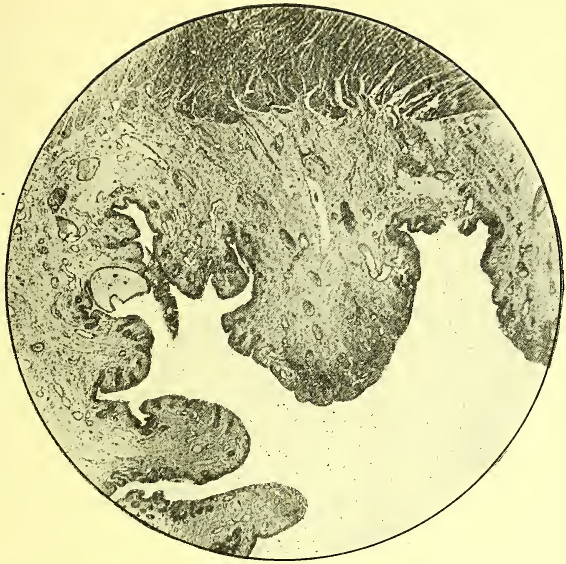

Fig. 9.—(×250.) Menstruating rabbit's uterus.

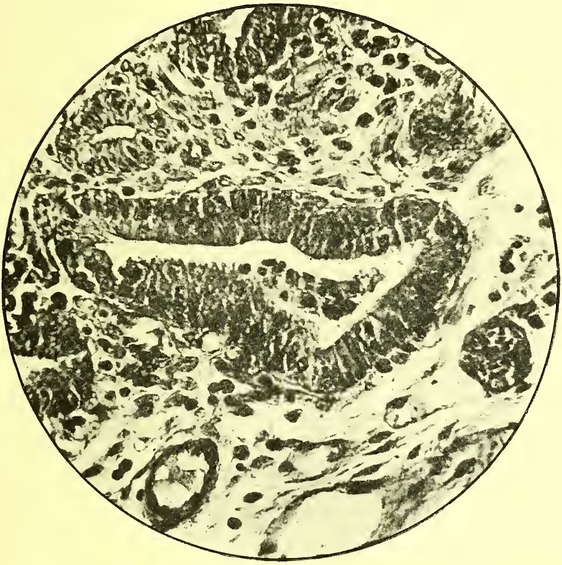

Fig. 10.—(× 1200.) Menstruating rabbit's uterus showing glandular activity.

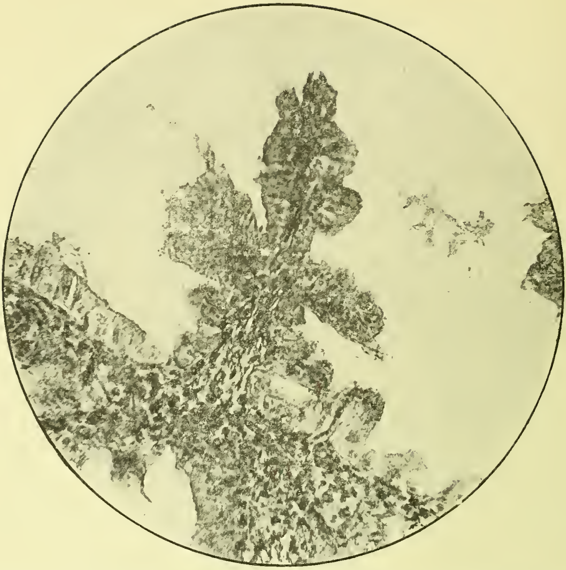

Fig. 11.—(×1200.) Menstruating woman's uterus showing glandular activity.

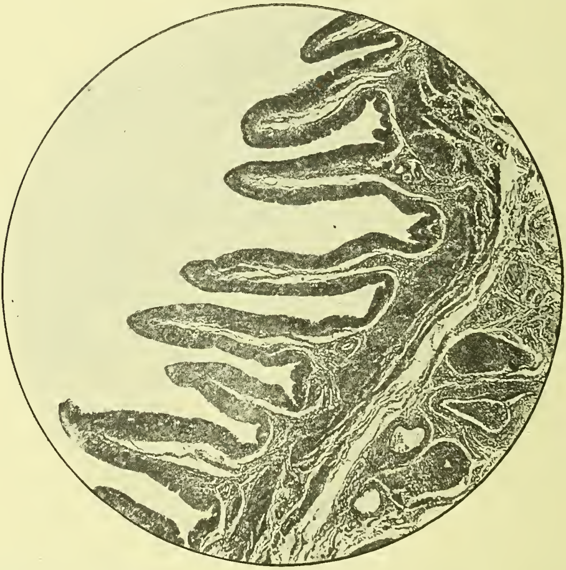

Fig. 12.—(×250.) Calcium-secreting chamber of a on-laying hen.

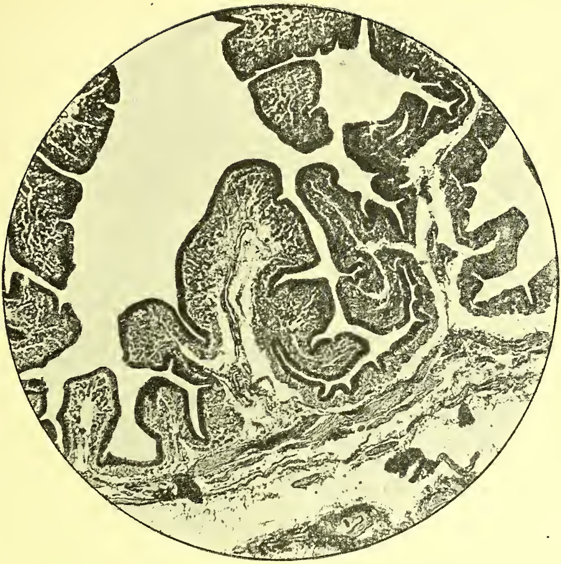

Fig. 13.—(×250. Calcium-secreting chamber of a laying hen.

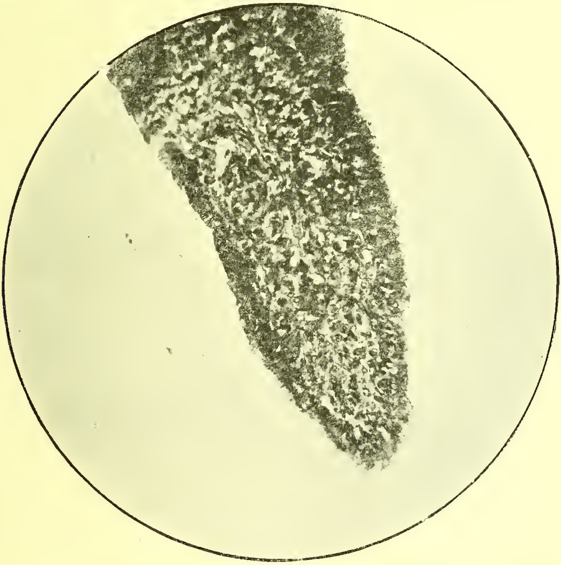

Fig. 14.—(×1200.) Tip of ruga from calcium chamber of non-laying hen.

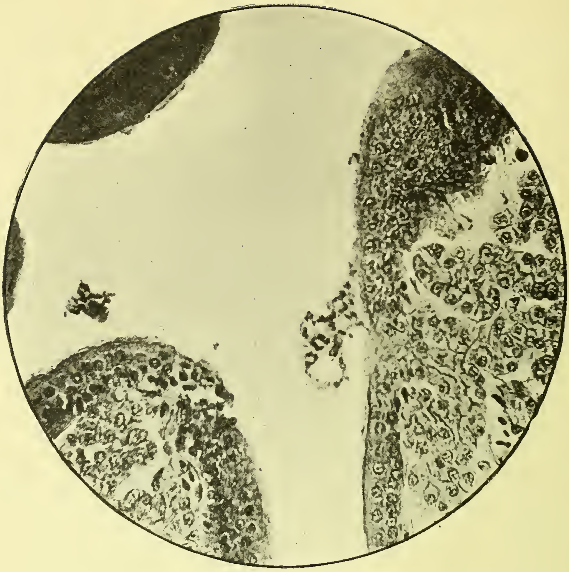

Fig. 15.—(×1200.) Rugae from calcium-secreting chamber of laying hen.

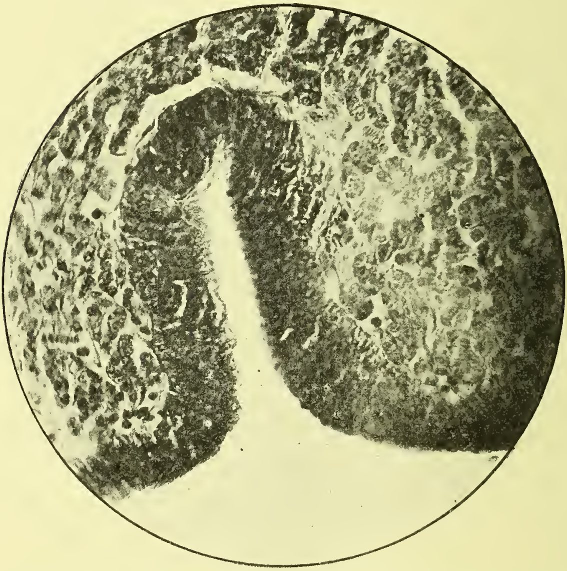

Fig. 16.—(×1200.) Superficial gland in calcium chamber of laying hen.

menstruation. These influences, apart from pregnancy,* are seasonal and climatic, dietetic and hygienic. How important they are can be gauged by the effect evolution has had, or even by the effect domestication has upon the habits of wild animals and civilization upon the human race.

The periodicity of the catamenia is, then, merely the result of a concomitant series of metabolic processes—such as are seen in other spheres of physiological activity—which may or may not allow of regular menstruation. We do not think we need go further into this point; it seems to us to be very obvious, and if viewed in this light makes a strong argument for our ideas concerning this influence of the calcium salts in particular.

As already mentioned, we have come to the conclusion that the calcium metabolism is largely influenced by the secretions of the ductless glands, and it may be worth while, therefore, to mention briefly the part which these secretions may play in the economy of this function. In the first place, let us point out that clinically we have used the calcium salts with very good results in the following classes of cases:

1. *Amenorrhoea* from general debility of a chronic nature, but more especially that resulting from acute diseases, such as influenza, in which the exhibition of calcium (in doses of 30 grains of calcium lactate every other day) not only relieves the amenorrhoea but also the symptoms ("nervous headaches," etc.) associated with the debilitated state of the patient.

2. *Menorrhagia* due to what are called "constitutional" conditions; that is to say, where there is no local pathological manifestation. We explain this paradoxical position in regard to amenorrhoea and menorrhagia in the following way: In the first class of cases there is not sufficient calcium at the disposal of the economy for menstruation, and in the second, that though there is enough for menstruation the blood content does not rise rapidly enough to stop the flow.

Now, it is a matter of common observation that, in young girls especially, the thyroid enlarges in the majority of cases just before the onset of menstruation—that is to say, when the blood calcium content is high. With the enlargement the onset of bleeding soon follows. Incidentally, it may be mentioned that a similar enlargement of the gland occurs *early* in pregnancy, when the menstruation is suppressed, and before the fetus can utilize the amount of calcium at its disposal in the blood. We have also observed this thyroid enlargement in artificial menopause.

The function of the thyroid secretion in this respect is to stimulate calcium excretion, which may take place from the uterus, or, failing that, from the kidneys or by the bowel. In this way thyroid may be a valuable drug—indeed, it has often been so used—in amenorrhoea.

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* The interesting fact, which has been overlooked or underestimated by most students of menstrual periodicity, of repeated oestrus (every fortnight in rabbits) in unimpregnated animals is surely comparable to the monthly menstruation of non-pregnant women. That this condition is not more frequently observed in animals is due to the promptness with which impregnation usually occurs.

In the same way, we believe that the internal secretion of the ovary has the property of depleting the blood and tissues of calcium salts, and possibly a special action in stimulating excretion by way of the uterus.

We have in opposition to these the secretion of the adrenals, and probably that also of the pituitary gland. Both, as will be shown in a subsequent paper, act in a similar manner upon the uterus, especially when in the pregnant state. But, as regards their influence upon menstruation, the extract of these glands assists in the production of uterine contractions which are marked at that time, and also in other more obscure ways from their relationship to calcium metabolism in general, and in particular that of menstruation.

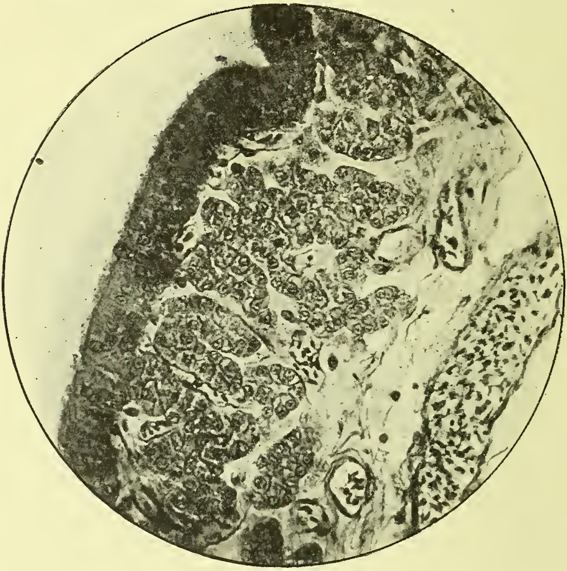

Fig. 17.—Vascularity and diapedesis in calcium chamber of laying hen.

Further, with regard to the secretion of the pituitary body—for we have at present little direct clinical evidence in regard to the adrenals—it has been shown that tumours of, and in the neighbourhood of, the pituitary gland cause amenorrhoea, and that cessation of the menses is one of the earliest symptoms of acromegaly.[9] It has also been shown that sexual infantilism with an absence of menstruation is a general complication in early disease of the pituitary gland.[10] An analysis of about 150 cases of acromegaly recently made confirmed the above statement regarding amenorrhoea, and also that lactation hastens the progress of the disease.[11]

CONCLUSIONS.

1. That menstruation is a periodic function only in so far as the calcium metabolism is in harmony with this periodicity, and that the function is dependent upon the calcium metabolism in all its ramifications.
2. That the haemorrhage into the Graafian follicle may be coincidental, and is probably the result of the lowered coagulability of the blood or vasomotor changes; but that rupture of the follicle is in no way responsible for menstruation.
3. That the bleeding from the uterus, while due to the lowered coagulability of the blood in part, is also dependent on the local changes in the capillaries from which the diapedesis of leucocytes and corpuscles occurs; and, further, that these leucocytes are an active factor in the conveyance of calcium salts from the glands to the exterior.
4. That the uterine glands excrete calcium and mucin, and that therefore the uterus is a " menstrual organ."[12]
5. That there is a correlation between the ovaries and uterus in reference to menstruation, but that the ovary is probably no more predominant than other ductless glands in this respect.
6. That menstruation *per se* is not a necessary adjuvant nor concomitant to fertility and reproduction.

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III.—THE CORRELATION OF THE UTERUS AND OVARIES.

In this paper we shall give the results (for the most part histological) of several series of experiments which bear upon the correlation of the ovaries and uterus, apart from the metabolic relations existing between these organs in regard to menstruation, which have already been discussed.

The experiments performed consisted of :

A.—1. Removal of ovaries and subsequent histological examination of the uterus.
  2. Removal of ovaries during pregnancy.
B.—Removal of uterus and subsequent examination of the ovaries.
C.—Ligation of uterine cornua :
  1. Without removal of ovaries.
  2. Synchronous with removal of ovaries.
  3. Subsequent to removal of ovaries.

The animals operated upon were for the most part rabbits. Three bitches were also used.

Most of the above experiments have been carried out by Bond, Marshall and other observers, but, inasmuch as there have been to some extent conflicting statements made in respect to the results obtained, we have been tempted to carry out these experiments ourselves in order that we might make up our own minds on several of the contentious matters, and on those points concerning which, on theoretical and clinical grounds, we have held views contrary to the results generally obtained. We may say briefly that for the most part our results correspond with those obtained by other observers, but we have been forced to the conclusion that the rabbit is not the best animal on which to carry out experiments, the results of which are estimated by histological examinations of the ovaries, if we wish to compare these results with conditions obtaining in the human subject. Indeed, we believe that certain questions of the greatest importance, such as the effect of the removal of the uterus upon the ovaries, will only be settled by prolonged experiments upon monkeys, or by clinical observation on the human subject; but of this we shall have more to say later. The fact, however, remains that the adult rabbit's ovary is of a very different histological structure from the dog's ovary or the human

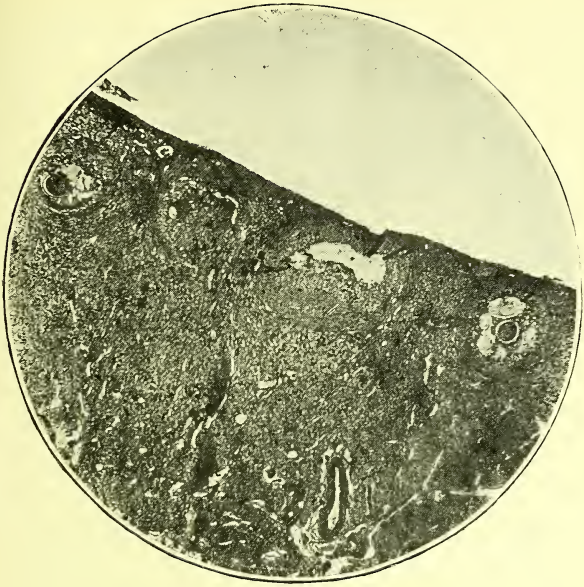

Fig. 1.—Adult rabbit's ovary. × 200.

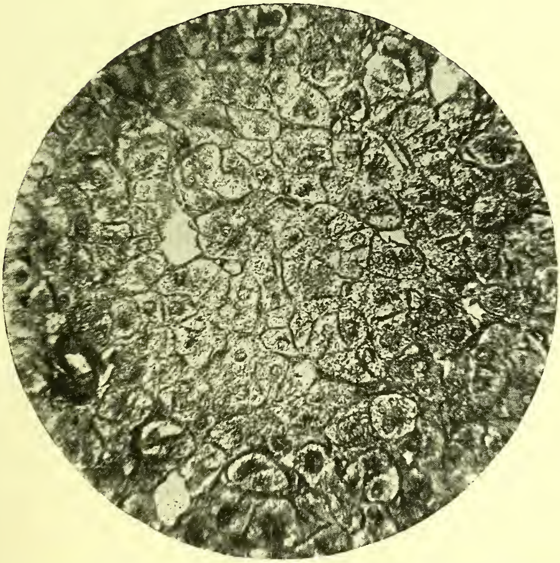

Fig. 2.—Adult rabbit's ovary showing interstitial (glandular) cells. × 600.

ovary. It has, however, been a matter of surprise to us that we have not come across adequate appreciation by those who have used rabbits in their experiments of the great difference between the histological structure of these ovaries and that of the human ovary. This difference only becomes marked in the adult rabbit, and may be summarized by the statement that in every normal rabbit—whether virgin or multiparous—the ovary is mainly composed of large cells, sometimes hardly to be differentiated from those which fill the corpora lutea, while there is practically no ordinary stroma. The ova themselves do not appear to be numerous, and are situated near the periphery. (See Fig. 1.) The adult rabbit's ovary, in fact, appears to be of an extremely glandular nature (see also Fig. 2), and in this respect entirely different from the dog's and the human ovary. (See Figs. 3 and 4 respectively.)

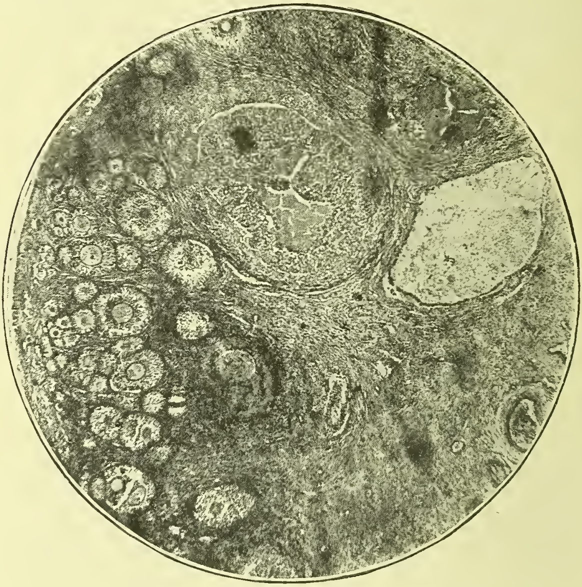

Fig. 3.—Bitch's ovary. × 200.

These large glandular "stroma" correspond, of course, with the "interstitial" cells which have been investigated by Miss Lane-Claypon.[1] In her opinion, based on careful histological work, these cells are derived from the germinal epithelium, as also are the ova and follicular cells. We have not conducted any detailed research into their origin, but it seems to us that this method of origin is a little strange, seeing that in the ovary of the young rabbit these interstitial cells are entirely absent or very few in number, while primordial ova seem to be more plentiful than in the adult (see Fig. 5).

Whatever their origin, there is no doubt that the presence of the interstitial cells makes the ovary a more glandular organ in the rabbit than in the human subject—the very friability of the adult rabbit's ovary being of itself evidence of a considerable difference of structure from that obtaining in most other mammals. Fraenkel[2] has recently made a long and important contribution on the subject of *la glande interstitielle de l'ovaire*, as it was originally called by Limon. After examining 45 species of mammals he concluded that these cells vary in extent in all, but are most marked in rodents. (We have found them constant in rabbits.) In his (Fraenkel's) experience he has found no trace of this glandular formation in the human subject. In view of these investigations by Fraenkel, and by ourselves in a very small way, we would suggest that the variation may be in proportion to the activity of the thyroid gland, which is notably inactive in rabbits.

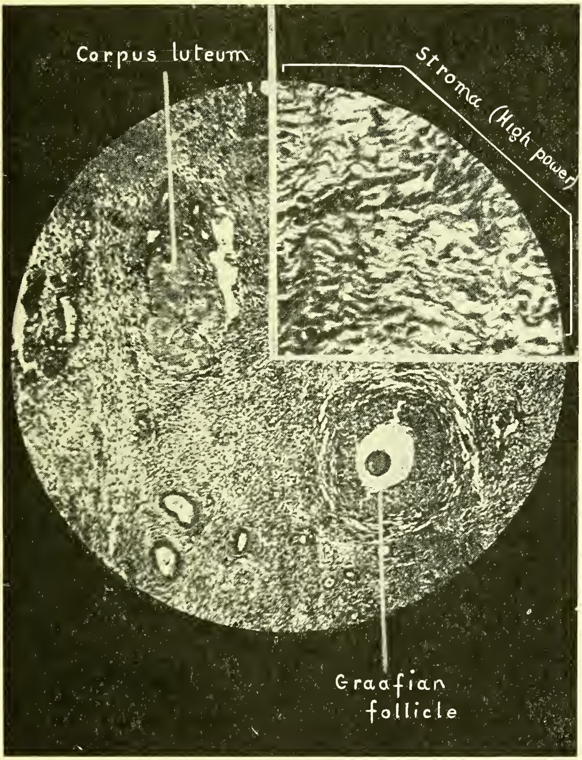

Fig. 4.—Human ovary (adult) × 200, with segment showing structure of the stroma × 600.

Attention has already been called to the close relationship which exists between the ovaries and the thyroid gland in regard to the menstrual function and to the calcium metabolism (see Papers I and II).

RESULTS OF EXPERIMENTS.

SERIES A.

I.—*Effect of Oöphorectomy upon the Uterus.*

In this experiment we wished to discover, if possible, whether the atrophy which is known to occur in the uterus after removal of the ovaries affects more the endometrium or the muscular layer. For the experiment we used one bitch and rabbits. The following are illustrative of the results obtained:

(*a*) Sable mongrel bitch, aged 6 weeks. Oöphorectomy performed. Animal killed and uterus removed nine and a half months later. It will be seen from Fig. 6 that there is a general arrest of growth which affects the whole uterus, and further that there is some degree of atrophy—most apparent in the muscle layer—which is readily seen on comparison with the uterus from a twin sister, removed on the same day that double oöphorectomy was performed on this bitch (see Fig. 7). Had the uterus, shown in Fig. 6, which was 10 months old, remained *in statu quo* after oöphorectomy, it should have resembled that shown in Fig. 7. There is, therefore, arrest of growth and then atrophy, and the latter most marked in the muscle layer.

(*b*) Doe, aged 6 weeks. Oöphorectomy. Animal killed two months later and uterus examined. A section is seen in Fig. 8. The same condition of affairs is seen in this as was evident in the last case. There is some atrophy of the endometrium, but want of development and atrophy are most apparent in the muscular layer. At one point there is an area of exceptional thinning. This is most unusual and is difficult to account for. In connexion with these two cases it may be contended that, owing to the fact that the uteri had never become active (see Paper IV), the muscle layer had in consequence not developed. This point will be further alluded to presently.

(*c*) Doe, full grown. Oöphorectomy. Animal killed and uterus examined three months later. A section of this uterus (see Fig. 9) shows that the changes which have occurred are most marked in the muscle layer, and that the endometrium is still apparently normal. This is better seen in the section than in the photomicrograph of it.

(*d*) Doe, full grown. Oöphorectomy. Animal killed and uterus examined three and a half months later. The condition alluded to is seen in this case most clearly (see Fig. 10). The endometrium is quite thick, while the muscular layer is represented by a very narrow band. This animal was pregnant when the ovaries were removed.

It may be of interest to mention here the case of a patient, aged 35, from whom the uterus was removed by one of us by operation (owing to extensive adhesions to the appendix, etc.). The ovaries had both been removed (by another surgeon) for dysmenorrhoea four years previously, which of course influenced us in removing the uterus. The organ removed was examined microscopically (Fig. 11). The glands exist in large

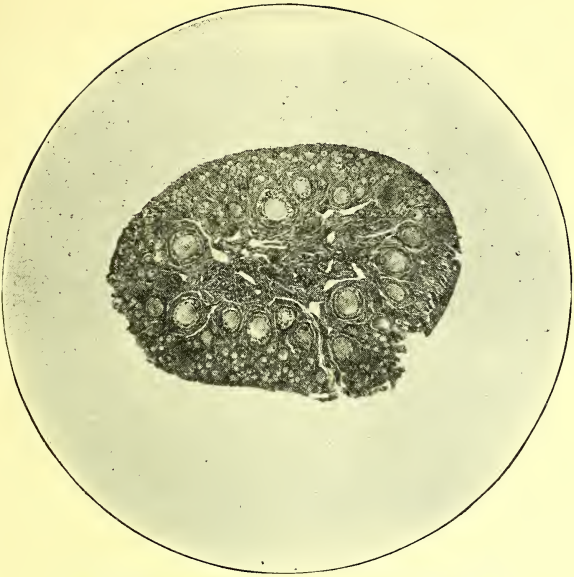

Fig. 5.—Ovary from rabbit a few weeks old. × 200.

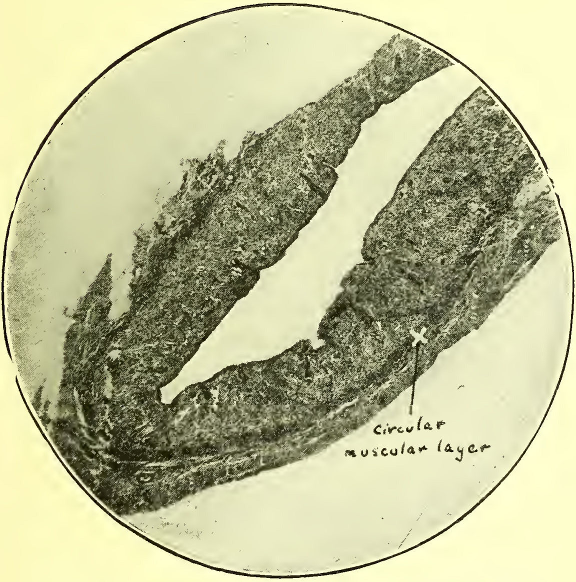

Fig. 6.—Young bitch's uterus nine and a half months after oöphorectomy. × 200.

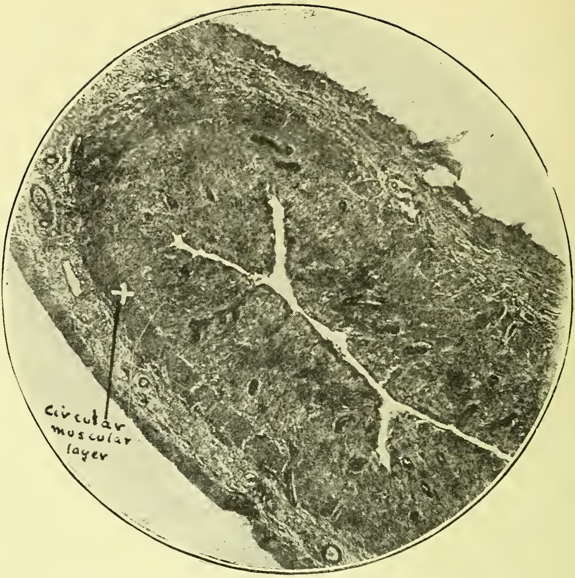

Fig. 7.—Bitch's uterus at age of 6 weeks. × 200.

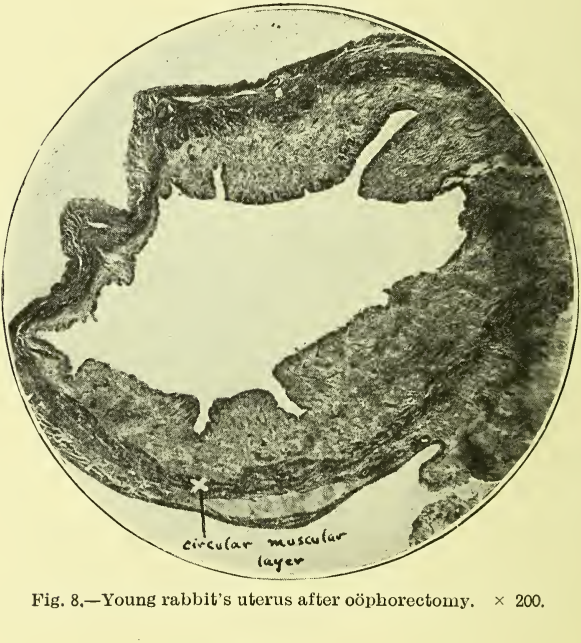

Fig. 8.—Young rabbit's uterus after oöphorectomy. × 200.

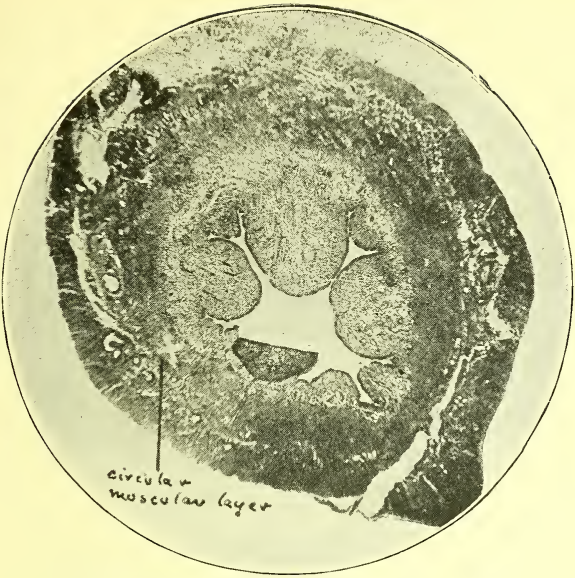

Fig. 9.—Rabbit's uterus after oöphorectomy. × 200.

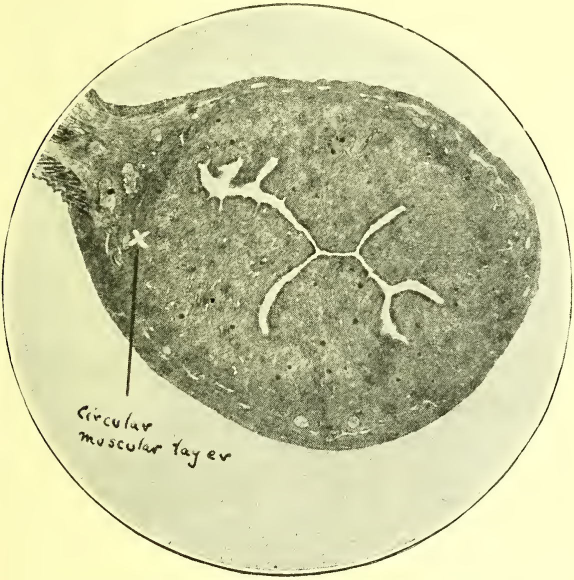

Fig. 10.—Rabbit's uterus after oöphorectomy. × 200.

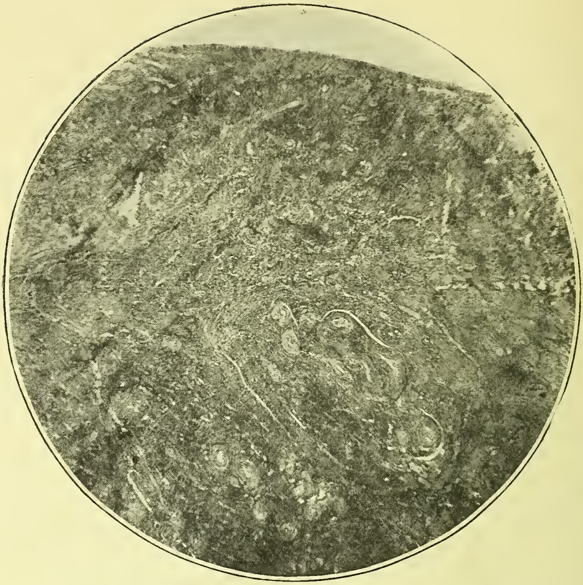

Fig. 11.—Uterus of woman four years after oöphorectomy. × 200

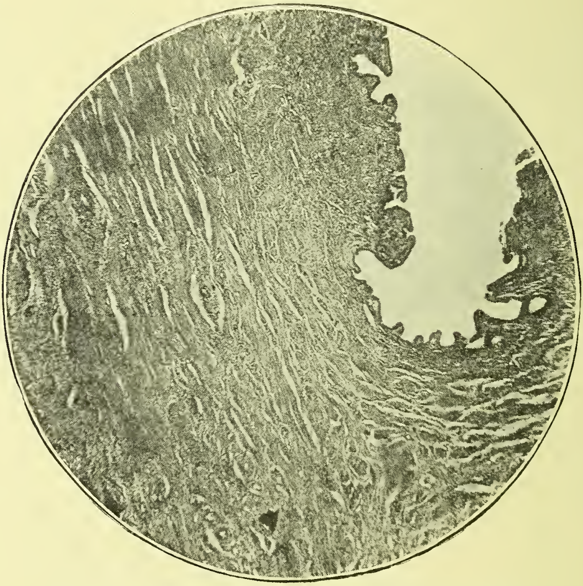

Fig. 12.—Infantile uterus from adult woman. × 200.

numbers and stain well. The delicate stroma of the endometrium and the muscle fibres are both replaced by fibrous tissue.

It would appear, then, that when the ovaries are moved in a young animal the uterus remains in an infantile condition, and undergoes slight general atrophy, which condition may be changed into one of general fibrosis of the organ after many years.

This condition of fibrosis is shown in Fig. 12, which is a section of an infantile uterus removed from a human adult. At the same time a rudimentary uterus may coexist with functionating ovaries, although this uterus may be a mass of fibrous tissue, as is seen in Fig. 13—a section of a rudimentary uterus removed by one of us from a hernial sac. (Owing to the extreme hardness, it was impossible to obtain a good section.) In this case the ovaries were proved to be active, or at any rate to contain corpora lutea on histological examination (Fig. 14).

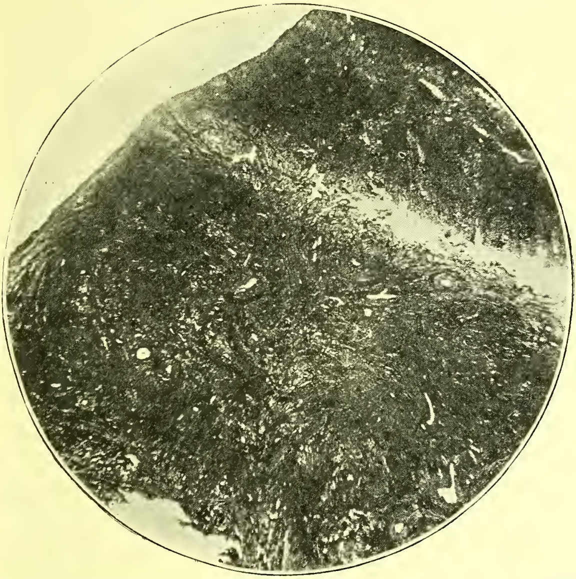

Fig. 13.—Rudimentary uterus from the hernial sac of an adult woman. × 200.

Conclusions.

1. Removal of the ovaries in young animals causes the uterus to remain infantile—atrophic changes eventually occurring.

2. Removal of the ovaries in adult animals produces *first of all an atrophic change in the muscular layers;* and this in time may cause atrophy of the endometrium, probably

by interfering with the circulation, the whole organ becoming invaded by fibrous tissue after sufficient lapse of time.

3. Functional ovaries may coexist with infantile uteri.

II.—*Removal of Ovaries during Pregnancy.*

In these experiments we endeavoured to remove the ovaries of rabbits at different stages of pregnancy as estimated from their connexion with the buck. The following are illustrative cases:

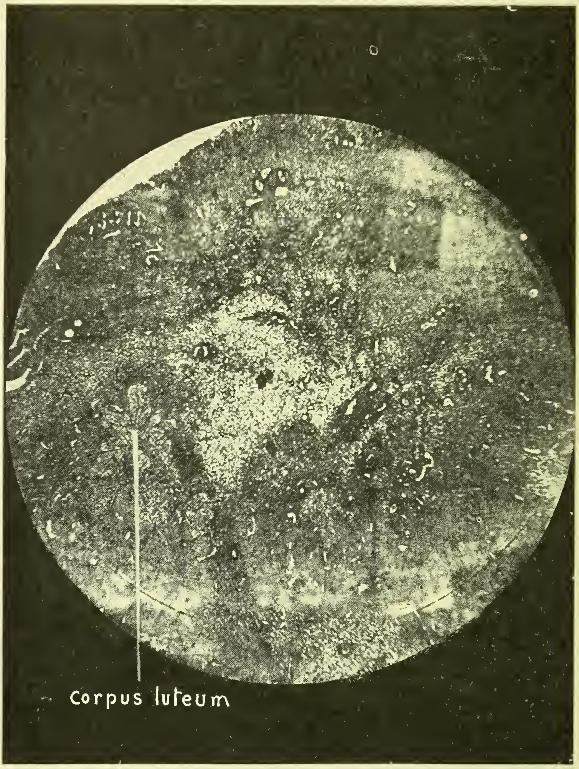

Fig. 14.—Ovary from hernial sac of an adult woman (same case as Fig. 13). × 200.

(*a*) Black doe. Opened in the anticipation that she was a few days pregnant. As pregnancy could not be detected, the ovaries and uterus were examined and handled and the abdomen closed. There was no further connexion with the buck. Twenty-three days later one young one was born alive. This case was therefore regarded in the light of a control experiment.

(*b*) Grey doe. Oöphorectomy was performed on the seventh day. The uterus was enlarged and small nodules visible. No abortion was seen to occur. On the thirty-third day after operation the abdomen was opened, and the uterine cornua were found to be very small. Abortion had undoubtedly occurred.

(*c*) Black doe. Oöphorectomy was performed on the fourteenth day of pregnancy. Abortion took place on the following day. The animal was killed twenty days later and the uterus found to be quite small.

(*d*) White and grey doe. Oöphorectomy was performed on the twentieth day of pregnancy. Abortion occurred on the following day.

Other similar experiments were performed, with the invariable result that abortion occurred in every case in which oöphorectomy was performed. Great care was taken in all cases not to expose the uterus, nor to handle it, and the operation was always completed in two or three minutes.

There has been much discussion in connexion with this question since ignipuncture was first performed upon the corpora lutea of pregnancy, and Fraenkel[3] claimed that the secretion of the corpus luteum was necessary for the implantation of the ovum. There are, however, still those who assert that the operative manipulations cause the abortion which usually occurs. We are inclined to think that it is not the operative manipulation altogether, in view of our control experiment; at the same time, we do not think that a comparison can be made between rabbits and the human subject in this respect, for there are undoubted cases on record in which the ovaries have been removed from women early in pregnancy (six weeks), and where there could be no doubt that the whole of both ovaries was removed, and yet the pregnancy continued to full time. A good example of this is the case reported by J. E. Gemmell,[4] with the subsequent history of which he has kindly acquainted us.

It would be fair, therefore, to say that while the internal secretion of the ovary is favourable to the continuation of pregnancy (and this will be easily understood by a reference to what we have already stated in connexion with the relation between the ovary and the calcium metabolism), on the other hand, this secretion is not absolutely essential in that respect—at any rate, in the human subject. That our experiments and those of others on rabbits have given positive results may be explained by a consideration of what has already been said in this paper as to the structure of the rabbit's ovary.

SERIES B.

Removal of Uterus and Subsequent Examination of the Ovaries.

On the whole, we have not been satisfied with the experiments we have conducted in this inquiry. We operated upon two bitches and twelve rabbits. All the animals were about 6 weeks of age at the time of operation. Unfortunately our bitches died of distemper within a few months of the hysterectomies, so that they never reached sexual maturity. We lost five of our rabbits, too from an epidemic of pneumonia. The rest (about half) that reached sexual maturity were killed at different periods, the longest time that was allowed to elapse between the operation and the examination being eight months. In every case the uterus was removed and the Fallopian tubes as far as practicable. In several cases, when the animals were killed, it was found, however, that the whole of the Fallopian tube had not been removed. It will be understood how difficult it was to effect this in view of the small size of the animals.

In no case did we find any histological change which could have been held to have occurred as the result of removal of the uterus. At the same time there appeared to be the negative effect that in no case could we discover the formation of corpora lutea,* or any evidence that Graafian follicles had ruptured, in spite of the fact that the animals came on heat and had connexion with the buck.

In Fig. 15 is seen a normal adult rabbit's ovary with a corpus luteum. In Fig. 16 is seen a section of an ovary removed from a doe whose uterus had been removed eight months previously, and who had frequently been to the buck. Many sections were cut of this ovary, but nowhere could we find evidence of corpus luteum formation. Ripe follicles and degenerated follicles there were in abundance, but nowhere was there any evidence that a follicle had ruptured. The degeneration, too, of the follicles appears to be of a more cystic nature than in the normal animal. It will be noticed in Fig. 16 that the interstitial cells are as well marked as in the normal ovary. In Fig. 17 is shown a section of the ovary from another doe whose uterus had been removed five months previously. In this case also no corpora lutea can be seen, although in other respects the ovary appears normal.

Heape[5] has produced evidence to show that the follicles in the normal rabbit's ovary do not rupture unless the doe has connexion with the buck. But in our cases the does that attained maturity had this connexion and apparently came on heat, but in spite of this there was no rupture of Graafian follicles. We must confess that we had fully anticipated that we should find marked changes in the ovary after hysterectomy in spite of the observations of others, and we based this belief on our own clinical experience and on the clinical experience of Abel, Zweifel, and others. But beyond the fact stated above—which may or may not be of importance†—we had no confirmation of our ideas. It is quite possible, however, that a much longer time must be allowed to lapse before changes become apparent, and once more we cannot refrain from saying that, in view of the structure of the

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* It is somewhat difficult to define what is meant by "corpora lutea" in rabbits' ovaries since there is no rupture of the follicle without connexion with the buck, but we may recognize *three* varieties (and we include all three in this term): True and false corpora lutea and the corpus luteum of normal degeneration.

† Especially in regard to lowered calcium content producing rupture of the follicle.—See Paper II. Compare also C. J. Bond, Experiment XXV (see references).

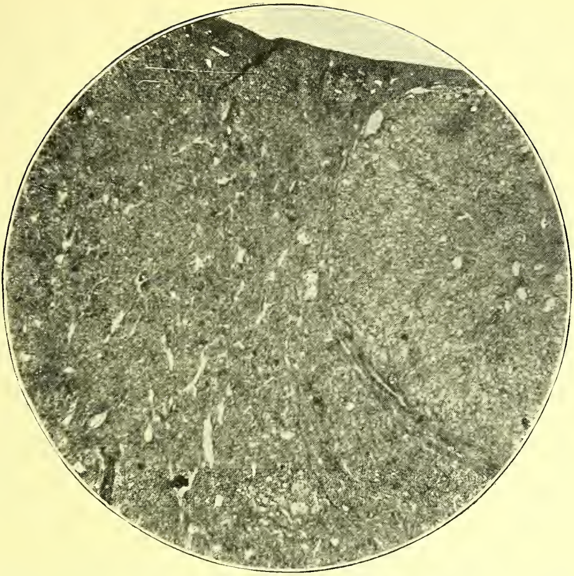

Fig. 15.—Normal adult rabbit's ovary with corpus luteum. × 200.

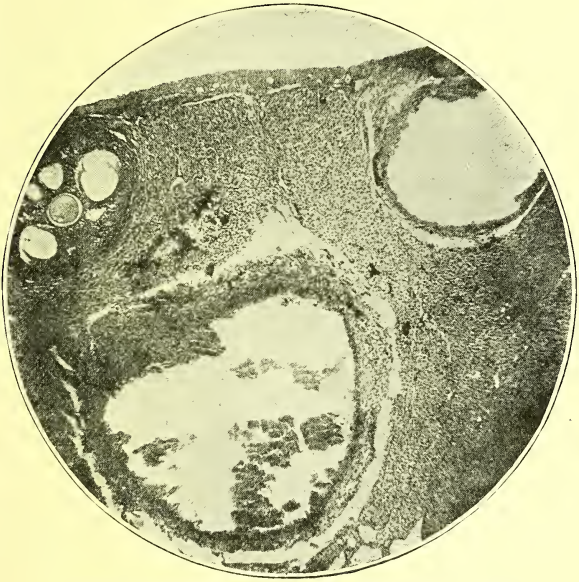

Fig. 16.—Rabbit's ovary after hysterectomy. × 200.

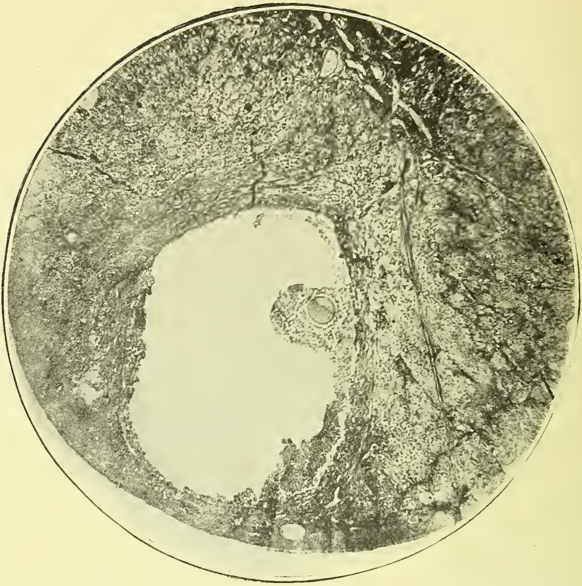

Fig. 17.—Rabbit's ovary after hysterectomy. × 200.

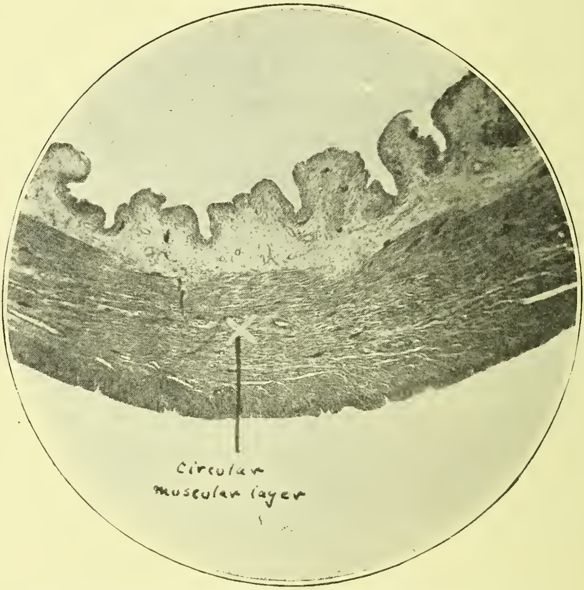

Fig. 18.—Hydrometra without removal of ovaries. × 200.

rabbit's ovary, comparison with the human ovary is hardly fair. We consider, therefore, that our experiments are inconclusive in this matter, and we shall hope to return to the subject at some future time.

SERIES C.

LIGATION OF THE UTERINE CORNUA.

In this series of experiments we have made observations on (*a*) the nature of the uterine secretion, (*b*) the condition of the uterus which had undergone expansion, (*c*) the condition of the ovaries (in the first experiment).

I.—*Ligation of the Cornua without Removal of Ovaries.*

We performed a large number of these experiments, in order to collect the fluid secreted by the uterus, for examination and experimental purposes. Consequently we were able to make a number of observations. In all cases we used silk as the ligature material, and usually we doubly ligated each uterine cornu, although it is not necessary to tie the ovarian extremity of the horn, as no leakage occurred in those cases in which we omitted to do so.

(*a*) *The Nature of the Secretion.*—This we have already dealt with in Paper II.

(*b*) *The Condition of the Uterus which had Undergone Expansion.*—A section of such a uterus is shown in Fig. 18. One observes a more or less even distension. The endometrium is flattened and thinned from pressure, but the muscular layers do not seem to suffer. It is probable that in those cases in which the muscular layers are well developed gradual distension of the uterine cavity leads to hypertrophy. This is a point of considerable interest, and very suggestive of the muscular hypertrophy which occurs in pregnancy. We intend to follow the matter up further.

(*c*) *The Condition of the Ovaries.*—We have been unable to trace any changes in the ovaries as the result of this experiment—microscopical or macroscopical. Bond thought that there were changes, but the condition that he found is probably merely the ordinary result of Graafian degeneration, frequently to be observed in rabbits that come on heat but do not have connexion. (Compare Heape, loc. cit.)

Figs. 19 and 20 are sections of young and mature ovaries respectively, removed from does in which hydrometra had been artificially produced. It will be seen that they are quite normal.

II.—*Ligation of the Cornua with Synchronous Removal of the Ovaries.*

(*a*) Fluid is freely secreted, the removal of the ovaries making no difference to the quantity. The fluid collected resembled that from the virgin uterus, containing less calcium salts than that obtained from a menstruating uterus. We also noticed that in its physiological effect it was not as active as that obtained from the menstruating uterus. (This will be referred to again in Paper IV.) In regard to this inactivity there was a possible source of error, in that it had been kept, sealed in glass capsules, for some little time before being used. We mention this fact though we are not ourselves convinced that it is one of any importance.

(*b*) In Fig. 21 is shown a section of a uterus the horns of which had been tied two and a half months previously, the ovaries having been removed at the same operation. In this particular case the rabbit had had young shortly before. Those experiments on rabbits that had not been recently pregnant gave the same results.

It will be seen from the section that a large hydrometra was formed, and that in the process of expansion the endometrium has become much thinned from pressure. It is interesting to note that *the muscular layer has not become atrophied to the extent it would have done had the uterine horns not been tied* when the ovaries were removed. We think that this is to be explained by the fact that the muscle fibres are called into use to resist the increasing pressure. This view is in harmony with the idea we hold that atrophy of the uterus after removal of the ovaries is primarily due to the consequent inactivity of the uterine muscle, which is, as we have already shown, the first portion of the organ to be a subject to degenerative changes. It is important to remember that this condition of muscular atrophy recurs seasonally in a physiological manner and degree in wild rabbits.

III.—*Removal of the Ovaries followed by Ligation of the Uterine Cornua at a Subsequent Date.*

(*a*) We found that the fluid secreted in these circumstances was as copious as in the last experiment, and closely resembled that secretion in its low calcium content and physiological inactivity. (This was also, however, as in the case of that obtained in the last experiment, kept some time in sealed capsules before being used.)

(*b*) A typical section of one of the uteri examined is seen in Fig. 22. In this case the animal had been pregnant, and had aborted after removal of the ovaries. Twenty days later the cornua were tied. A month later the animal was killed. In every particular the section of this uterus is comparable with that seen in Fig. 21, and in marked contrast with Fig. 10.

Removal of the ovaries, therefore, whether performed previous to ligation of the cornua or synchronous with that operation, does not affect the quantity of the secretion, while it does appear to alter the chemical nature of it, as already indicated by the lowered calcium content and reduced physiological activity.

The histological appearances of the uteri are striking and suggestive, and readily bear the interpretation already put upon them in regard to the hypertrophy rather than atrophy of the muscular layer under these conditions.

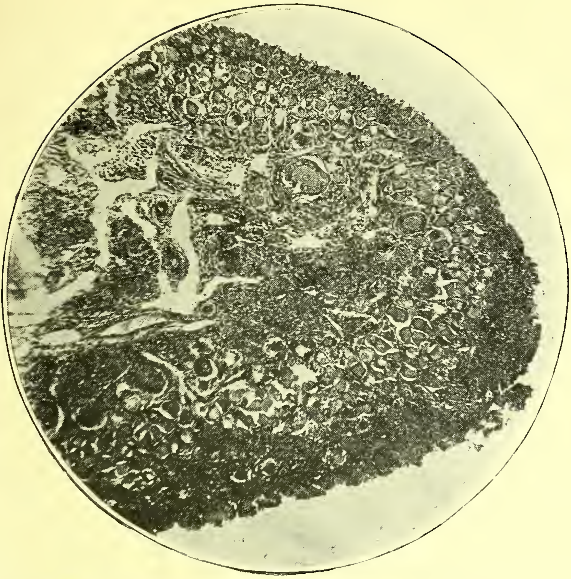

Fig. 19.—Ovary of young doe with hydrometra × 200.

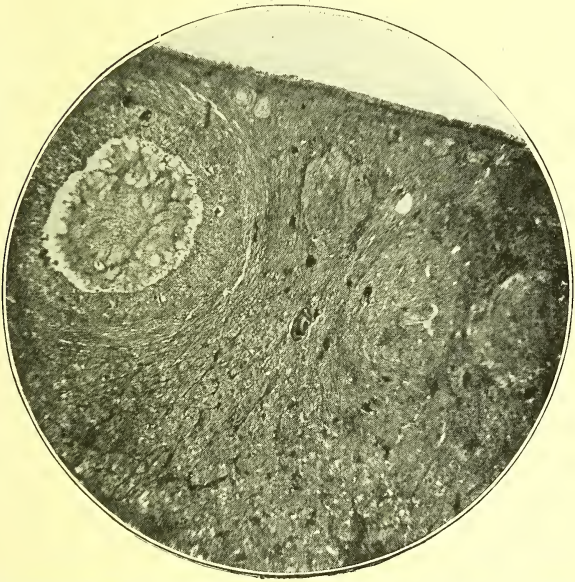

Fig. 20.—Ovary from young adult rabbit with hydrometra. × 200.

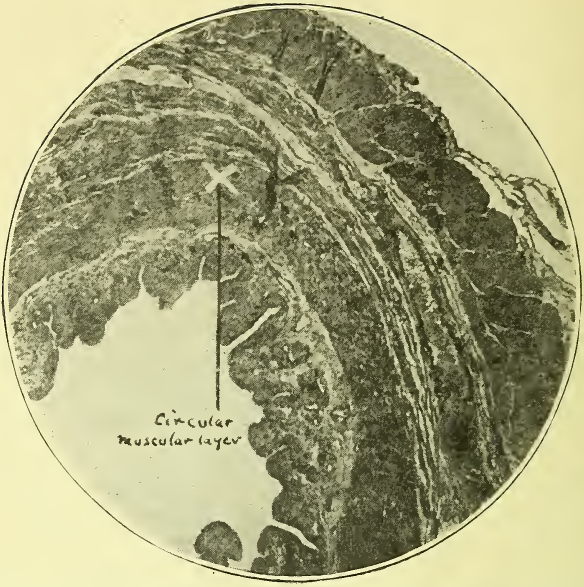

Fig. 21.—Hydrometra with synchronous removal of ovaries. × e200.

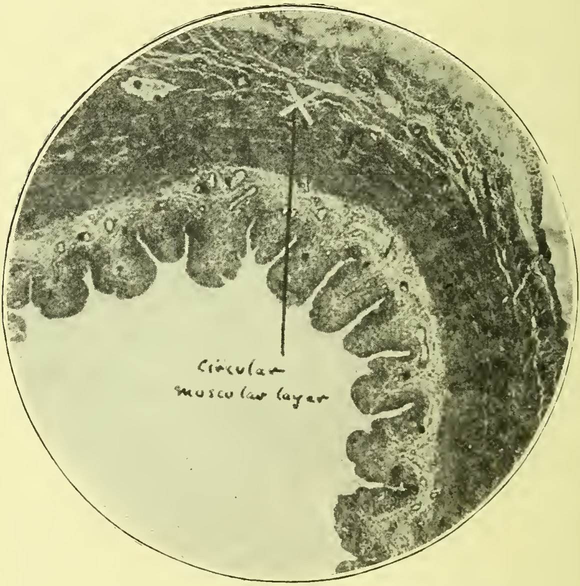

Fig. 22.—Hydrometra after previous removal of ovaries.

We found also in the course of our experiments on hydrometra that unilateral ligation of a cornu does not at all interfere with pregnancy in the other cornu. One of our rabbits in which we made a unilateral hydrometra and stitched it to the abdominal parietes for satisfactory observation subsequently had two litters, both of which appeared at full time, and were reared. We thought, however, that the cyst got smaller during the pregnancies. This bears out Bond's observations as to the absence of secretion from a cornual fistula during pregnancy in the other horn. Indeed, many of our experiments here recorded confirm much of what he has stated in a recent publication. It is only right, however, to mention that our experiments were begun before his paper appeared.[6]

There is one more experiment (for the successful performance of which we are indebted to Mr. James Smith) to which we would like to allude:

> One of us arranged with a keeper in Scotland to catch forty wild doe rabbits in February. Twenty of these were immediately killed and the genital organs examined. In every case the uterus was in the inactive or non-contracting state, and was flat and ribbon-like. The ovaries were small, and contained no ripe Graafian follicles. The remaining twenty rabbits were kept in captivity away from bucks for about two months, during which time two died. As far as possible the animals kept in captivity were allowed to lead a natural life, except for the presence of bucks. At the time these were killed and examined the ordinary wild rabbits were breeding. On examination every uterus was found to be in an inactive condition, and the ovaries were all small, and contained no ripe follicles.

It is, of course, possible, that captivity was the cause of this—of the fact that none were on heat; but we incline to the view that it was—in part at least—because the presence of the buck is an exciting factor in the case of wild does in regard to the onset of genital activity early in the year. We were quite unprepared for this result of our examinations, for we hoped to get inactive uteri in the first lot and menstruating uteri in the second, in order to estimate the total calcium in the ash of each lot of uteri. We give this observation for what it may be worth. The great difference between wild animals and domestic animals in their breeding habits is at once a source of wonder and information, and enables us more than anything else to realize how intimate is the association between the genital functions and the general metabolism of the body. The experiments we have described not only indicate that there is this close connexion between the general metabolism and the genital organs, but also that there is a definite interdependence (so long known and recognized, but never defined) of each part of the genital apparatus—that is to say, of the uterus and ovaries.

And, finally, these experiments help us to understand the specific changes that occur in each part as the result of interference with the other, or in altered circumstances. They have, in short, enabled us to attempt to define what that specific relationship is, and in coming to our conclusions we wish to acknowledge the assistance we have received from the work of those other observers in the same field of investigation to whom we have already referred.

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IV.—UTERINE CONTRACTIONS.*

Since uterine muscle consists of involuntary muscle fibres, one may take it as an accepted fact that most of those chemical substances which cause contraction of other involuntary muscle fibre throughout the animal body, whether produced by metabolic process normally or in specific circumstances or by experimental and therapeutic means, will also cause uterine contractions.

The object of our investigation was to discover what substance or substances, which might be said to exist in the blood normally, influenced the contraction of the uterus in respect to the ordinary contractions which are supposed to occur in the unpregnant uterus, and also those of the pregnant organ. In all our experiments we used rabbits, and our technique was as follows:

The rabbit was anaesthetized with ether, and, when fully under the influence of this anaesthetic, laid upon a heated table. The trachea having been exposed, tracheotomy was performed, and a tube connected with an electric bellows tied in position. The skull-cap was then trephined and the rabbit pithed, the brain being entirely destroyed, and the cranial cavity packed with wool plugs saturated with hot saline solution.

The abdomen was next opened with a 1-in. incision and the uterus and vagina identified. A small incision was made in the posterior wall of the vagina, close to the insertion of the cervices, and a special glass cannula, with a rubber-covered neck (see Fig. 1), connected with a one-arm water manometer, was carefully passed through the vulva, up the vagina, through one of the cervices, and into a uterine cornu. This was easily accomplished by directing the progress of the cannula through the small incision in the vagina. Before being passed into the uterus saline solution was allowed to flow through the cannula in order to prevent the entrance of air.

As soon as the head of the cannula was well in the uterus a soft worsted ligature was tied firmly round the cervix above the vagina, binding it against the rubber-covered neck. On one or two occasions elastic was used as the ligature material. The uterus was then returned to the abdominal cavity, placed in a normal position, and the abdomen closed. Enough saline was allowed to flow into the uterus to distend it slightly, but only so far as to support a column of saline solution 1 in. or 2 in. in height in the single-arm manometer. This manometer was next connected with a special bellows apparatus, very kindly made for us by Professor T. G. Brodie (see Fig. 2). This apparatus consists of a membrane finger-cot attached to a glass tube which is passed through a cork, the front of which is cut at an angle. To the lower end of this a card lip is attached to support the finger-cot. A card lid with a membrane hinge rests upon the protruding rounded end of the finger-cot. To the centre of this lid is fixed a piece of cork, to which a straw lever is attached by means of a needle. A glass writing point swinging from the distal end of the straw records the uterine contractions upon smoked paper revolving on a Brodie's kymograph apparatus, for it will be clear that as the uterus contracts the saline solution is driven up the manometer tube and the finger-cot distended. In all tracings the writing points were travelling from *right to left*, as indicated by the arrows below the blood pressure tracings.

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* The experiments recorded in this paper were carried out in the Physiological Laboratory in the University of Liverpool, and we are much indebted to Professor Sherrington for the facilities afforded.

The carotid artery was next connected with the mercury manometer, and the blood pressure recorded. The time was marked in seconds by means of an electric connexion with a clock.

The reagents used were in all cases injected intravenously, and the smallest doses compatible with a definite result employed. Control injections of simple saline solutions were frequently used.

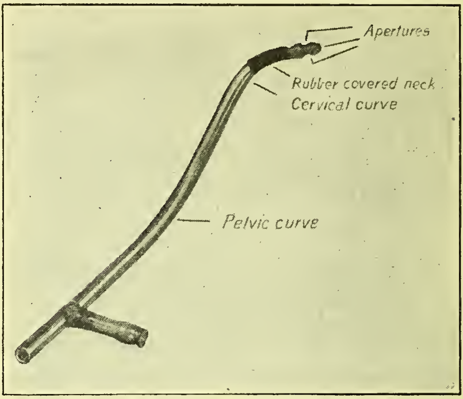

Fig. 1.—Uterine cannula.

It may be urged that pithing produces a condition of intense shock and low blood pressure. This, of course, is true; at the same time it is probable that the blood pressure is acted upon in much the same way, if in less degree, under these conditions by the reagents we employed, as would have been the case had pithing not been performed. Certainly pithing seems to have little effect on uterine contractions. On the other hand, we found it absolutely impossible to get satisfactory results under ordinary anaesthesia, owing to the variations in blood pressure, and the irregularity of uterine contractions—probably due to the impossibility of keeping the animal all the while in the same state of anaesthesia.

In regard to our method of recording uterine contractions, we consider it the most perfect yet utilized, in that *alterations of the total uterine content only are recorded ;* and, further, that the uterus is acting under practically normal conditions—the slight stimulus of saline solution under a low pressure being negligible from a practical point of view. The methods used by Cushny,[1] Dale,[2] and others are open to the objection that the uterus is exposed —even though it be in a bath—and, further, that isolated muscular contractions only are recorded, instead of alterations in the total content. Now we have found that contractions of the uterus may occur without the uterine content being altered at all. That is to say, the uterus may relax at one part while contracting at another. In our opinion the total alteration in the content, or, in other words, the expulsive force of the uterus, is the most important result of uterine contraction, and it was this we wished to measure; hence our employment of the method already described.

Before passing on to a consideration of the results obtained, we would like to point out, in addition to the fact just mentioned of peristaltic contraction, in which a relaxation compensates a contraction, that we have found the contractile power of different uteri to vary enormously according to the size of the rabbit and its age. In regard to the former point, it will be noticed that a great variation occurs in the size of the contractions in the different tracings. This is almost entirely dependent, subject to the conditions to be mentioned directly, on the size of the uterus, or, in other words, on the age and size of the rabbit. In addition to these factors, we must also point out, however, that the non-menstruating and non-pregnant uterus does not normally contract at all (see Fig. 3 and subsequent figures of inactive uteri). We have reason to believe that this applies to the uteri of wild rabbits in winter months (see Paper III). We shall for the sake of brevity refer to these as "inactive" uteri.

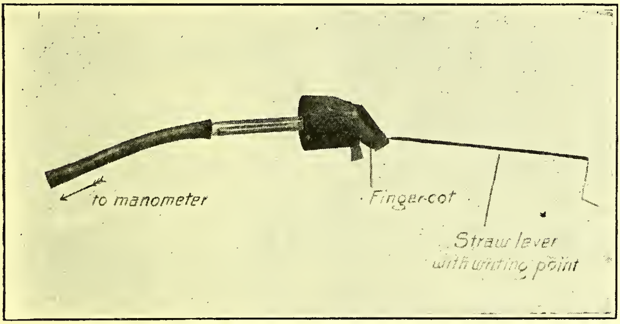

Fig. 2.—Recording bellows.

The menstruating uterus appears to be in a state of more or less regular expulsive contraction (see Fig. 4), and likewise the pregnant uterus (see Fig. 5), once the uterus when in this condition is interfered with. Unfortunately it is impossible to measure contractions without some interference, but we obtained conclusive evidence in one instance, in which the sacs were unruptured, that leads us to believe that the pregnant uterus does not normally contract expulsively. That is to say, any contractions which normally occur are compensated by relaxation at the same time. Our tracings of the contractions of the pregnant uterus are, therefore, from those experiments in which the membranes had been ruptured.

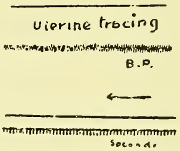

Fig. 3.—Inactive uterus showing no contraction. (Reduced about one-half.)

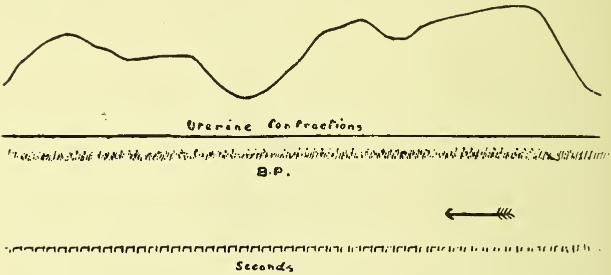

Fig. 4.—Menstruating uterus showing normal expulsive contractions. Note—Drum revolving faster than in Fig. 5 and in most of the other tracings. (Reduced about one-half.)

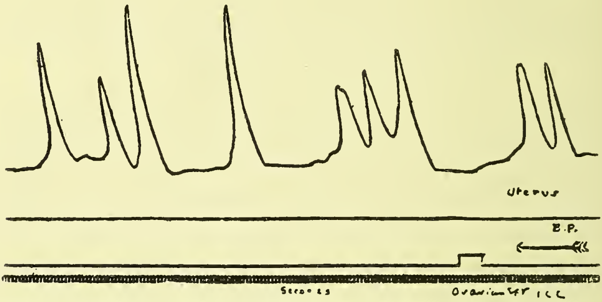

Fig. 6 (*continued*).—Menstruating uterus: effect of ovarian extract in repeated doses. (Reduced about one-half.)

In our experiments we have used all three varieties of uterus: The pregnant, the menstruating, and the virgin non-menstruating.

We have tried the effect of the following reagents upon these uteri by intravenous injection:

1. Ovarian extract.
2. Thyroid extract.
3. Pituitary extract.
4. Adrenal extract.
5. Placental extract.
6. Uterine secretion.
7. Calcium salts.

In general, we may say that in some cases the virgin non-menstruating uterus was affected, but that in those cases where any contraction was obtained it was more marked in the case of the menstruating uterus, and most marked in the pregnant uterus. That is to say, a uterus that can be caused to contract by any reagent reacts more readily and violently during menstruation and pregnancy.

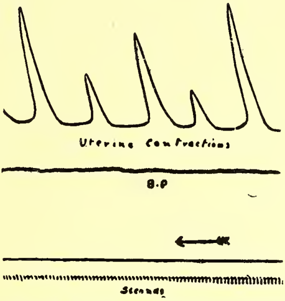

Fig. 5.—Pregnant uterus showing normal expulsive contractions. (Reduced about one-half.)

We have, in short, been unable to obtain those paradoxical effects, noted by some observers using different methods, of relaxation in the non-pregnant and contraction in the pregnant uterus.* Such results seem foreign to what one would expect, and are not, as we say, borne out by our results. In most of the experiments we were also able to note the effect of our reagents upon the blood pressure.

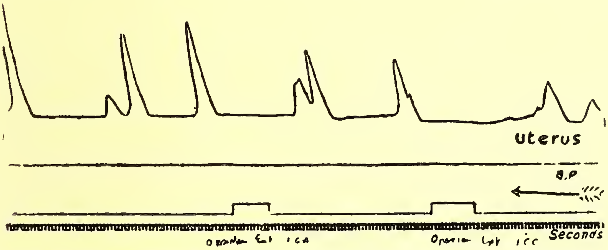

Fig. 6.—Menstruating uterus: effect of ovarian extract. (Reduced about one-half.)

1. *Ovarian Extract.*

The ovarian extract used was kindly prepared for us by Mr. H. H. Dale, of the Wellcome Laboratories. It was made by extracting fresh sheep's ovaries with cold saline solution containing a little chloroform. The extract was filtered first through paper, and then under pressure through a Berkefeld candle. A crystal of thymol was added to act as an antiseptic; 1 c.cm. of this extract contained 0.25 gram of fresh ovary.

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* Verbal communication, Dr. F. E. Taylor.

(*a*) This extract has no effect upon the inactive uterus.

(*b*) Upon the menstruating uterus the effect is well marked, as illustrated by the gradually increasing effect of a series of intravenous injections of 1 c.cm. of the extract This is shown in Fig. 6. The action is not excessively energetic, but still it is quite evident. Fig. 7 illustrates the effect in another rabbit, whose uterus was also menstruating. In this case a more or less tetanic spasm was produced.

(*c*) The pregnant uterus is affected in a somewhat remarkable manner by ovarian extract. The strength of the contractions are in no way lessened, but the immediate effect of an injection appears to be the stopping of the impending contraction. This is shown in Fig. 8, in which only one injection is illustrated. A subsequent injection produced the same effect, but with a slightly shorter interval, and a third injection hardly showed the effect at all, antibodies being probably formed in the blood by that time. We may say, however, that in the case of other pregnant uteri an increase in the strength of the contractions only was observed.

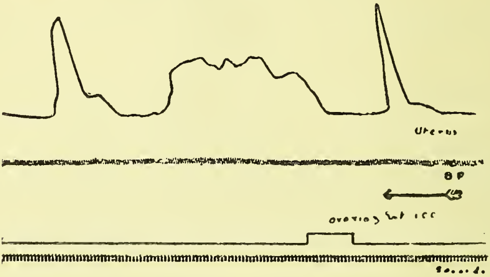

Fig. 7.—Menstruating uterus: effect of ovarian extract. (Reduced about one-half.)

The effect of the ovarian extract upon the blood pressure is hardly noticeable; if anything, there is a tendency to a gradual, uniform decline, as is shown in the lessening of the force of the heart-beats, and gradual progression towards the abscissa in Fig. 8.

2. *Thyroid Extract.*

For these experiments we used a solution of thyrocol of a strength of 1 in 10 in normal saline.

(*a*) There was no effect upon the inactive uterus.

(*b* Upon the menstruating uterus the effect of thyrocol was to cause an increase in the strength and number of uterine contractions. In Fig. 9 this result is seen after one injection. The results of a subsequent injection into the same animal are illustrated in Fig. 9 *continued*, where a general convulsion was noted after the third injection.

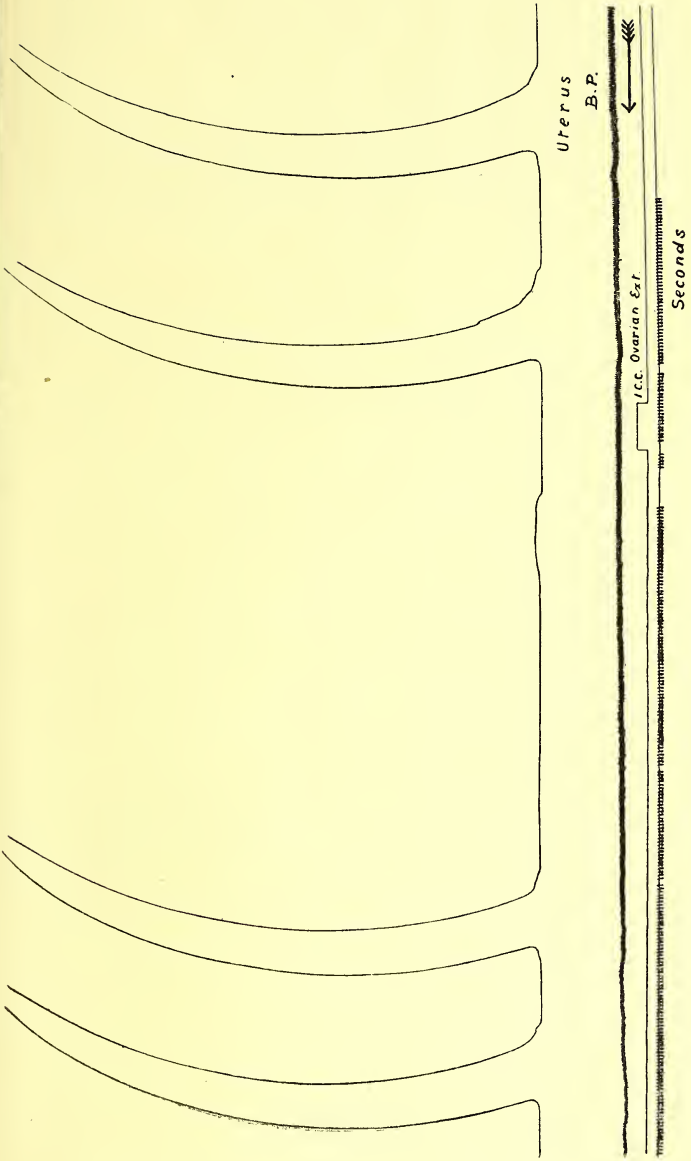

Fig. 8.—Pregnant uterus : effect of ovarian extract. (Reduced about one-half.)

(*c*) On the pregnant uterus the effect is the same as that upon the menstruating uterus, only more marked.

The effect of thyroid extract upon the blood pressure is shown in Fig. 9. There is always a distinct drop; this may be followed by a rise with the onset of a convulsion (see Fig. 9 *continued*)

3. *Pituitary Extract.*

The extract used was, in this case, also kindly prepared for us by Mr. H. H. Dale, of the Wellcome Laboratories; 1 c.cm. of the solution contained 0.1 gram of fresh infundibulum. As previously stated (see Paper I), it is only this portion of the pituitary gland which is active in the respect shown here.

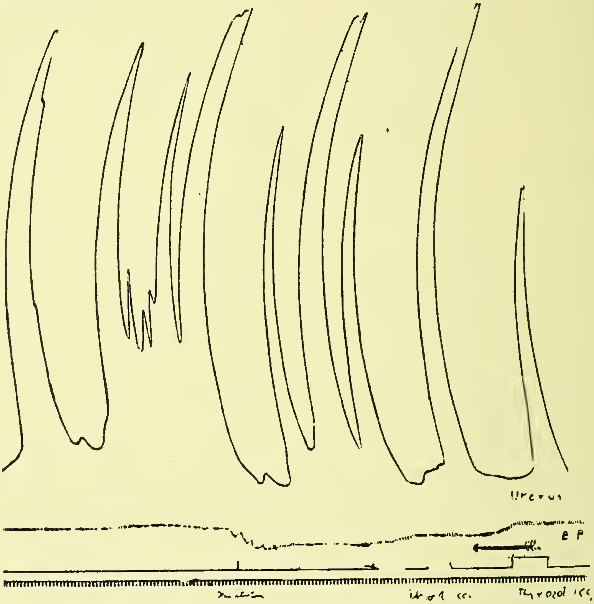

Fig. 9 (*continued*).—Menstruating uterus: effect of thyroid extract. (Reduced about one-half.)

(*a*) Upon the inactive uterus pituitary extract will produce a slight effect. This is shown in Fig. 10, where an evident wave of contraction is seen to occur in a uterus which had not previously contracted.

(*b*) Upon the menstruating uterus pituitary extract produces a very marked effect, causing much more powerful, frequent, and prolonged contractions than those occurring normally.

(*c*) Upon the pregnant uterus the extract produces the same effect as upon the menstruating organ, with the qualification that the contractions are, as always obtains in this condition, more forcible because of the increase in the muscular structure in the walls, and the greater sensitiveness to stimulation which is exhibited during pregnancy. This effect is shown in Fig. 11.

In Figs. 10 and 11 the effect of pituitary extract upon the blood pressure is well seen. There is an immediate and extensive rise. This rise is maintained for a considerable time. In Fig. 10 secondary rises—three in number, with two less important ones—are very prettily shown. These occurred during the act of defaecation, brought about, no doubt, by the effect of the pituitary extract on the intestinal muscles.

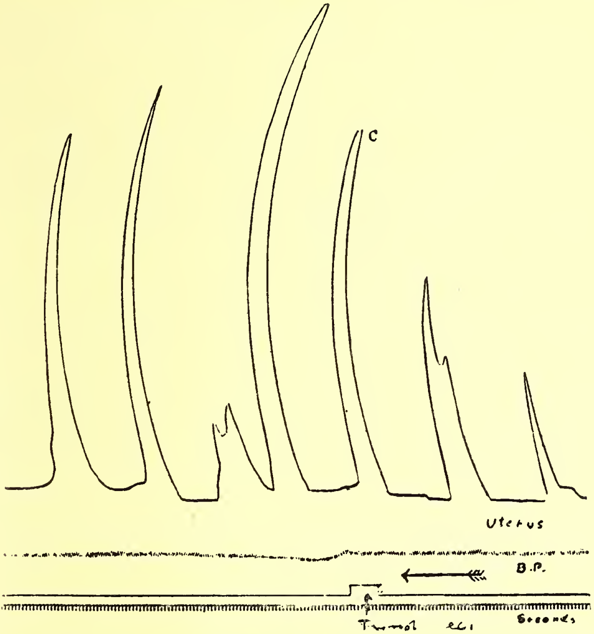

Fig 9.—Note: The contraction marked *c* followed the injection, the uterine marker being a little behind the proper line in this figure and in Fig. 9 *continued.* (Reduced about one-half.)

Mr. H. H. Dale also has shown the effect of this extract upon the uterus, using a different method.

4. *Adrenal Extract.*

The solution of 1 in 1,000 adrenalin prepared by Parke, Davis and Co. was used.

The effect upon the pregnant uterus is well known, consequently we only tried the effect of the extract upon the inactive uterus. The result is shown in Fig. 12. A distinct contraction was produced, together with the well-known rise in the blood pressure. This tracing is, in fact, almost identical with that shown in Fig. 10.

5. *Placental Extract.*

The preparation used was that kindly supplied to us by Dr. F. E. Taylor, who, with Professor Dixon, has recently published the results of their work with the extract prepared by them from the placenta.

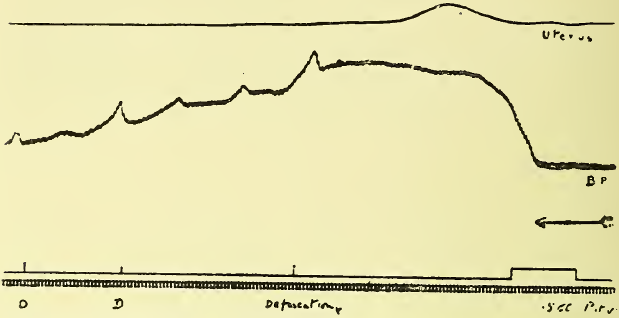

Fig. 10.—Inactive uterus: effect of pituitary extract (0.5 c.cm.). (Reduced about one-half.)

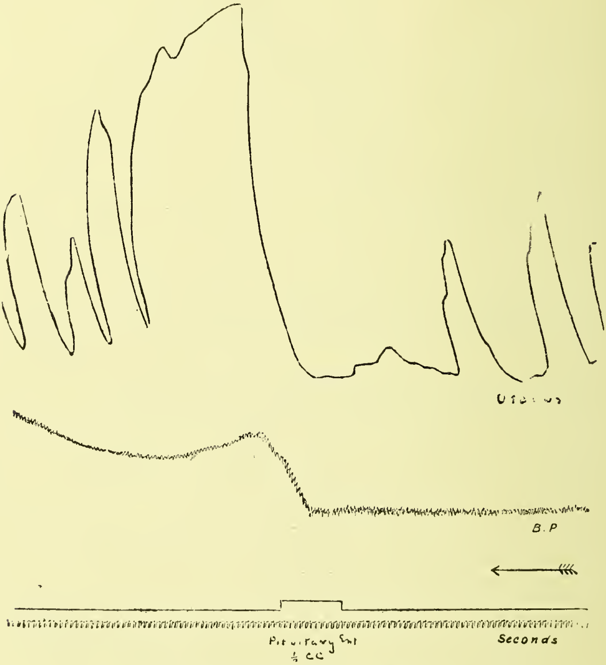

Fig. 11.—Pregnant uterus: effect of pituitary extract (0.5 c.cm.). (Reduced about one-half.)

(*a*) Upon the inactive uterus this preparation, used according to the instructions of Dr. Taylor, produced quite a marked effect, as is seen in Fig. 13, where two distinct contractions occurred as the result of a single dose.

(*b*) Upon the menstruating organs there appears to be a marked effect. The contractions are prolonged, and recur more rapidly, but their total expulsive force is not increased. This is shown in Fig. 14. It is possible the dose used (1 c.cm.) was rather small to produce a very decided result. Upon the blood pressure the effect is not at all marked, but there is a slight tendency for it to rise.

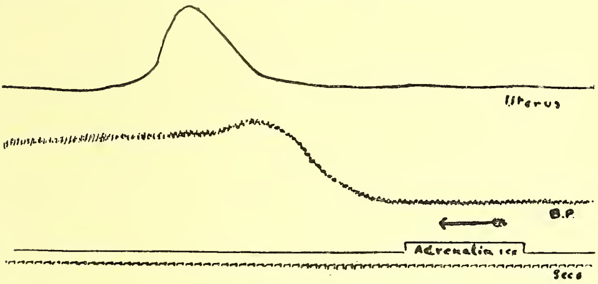

Fig. 12.—Inactive uterus: effect of adrenalin (1 c.cm. of 1 in 1,000 solution). (Reduced about one-half.)

(*c*) Our supply having run short, we did not test the action of this preparation upon the pregnant organ, but in view of our other experiences we have no doubt that powerful contractions would be produced.

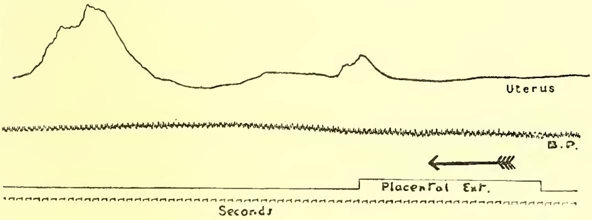

Fig. 13.—Inactive uterus: effect of placental extract (1 c.cm.) (Reduced about one-half.)

6. *Uterine Extract.*

In order to obtain this extract it occurred to us to use the secretion obtained from the uterus by ligation of the uterine cornua in rabbits. In this way we obtained, in a concentrated form, the secretion of the glands, and it may be taken for granted, therefore, that such a secretion would resemble in its action an active extract prepared from the whole organ. We found that the most active preparation was that obtained fresh and from a rabbit capable of menstruating. The secretion so obtained was very fatal in its effect unless used dilute. We used it, therefore, in a dilution of 1 to 4; even then we could readily produce fatal effects. Our records only show, of course, those cases in which no bad result, beyond perhaps clotting in the blood-pressure cannula, was produced. We found this secretion the most powerful in its effect upon the uterus of any of the animal extracts used by us.

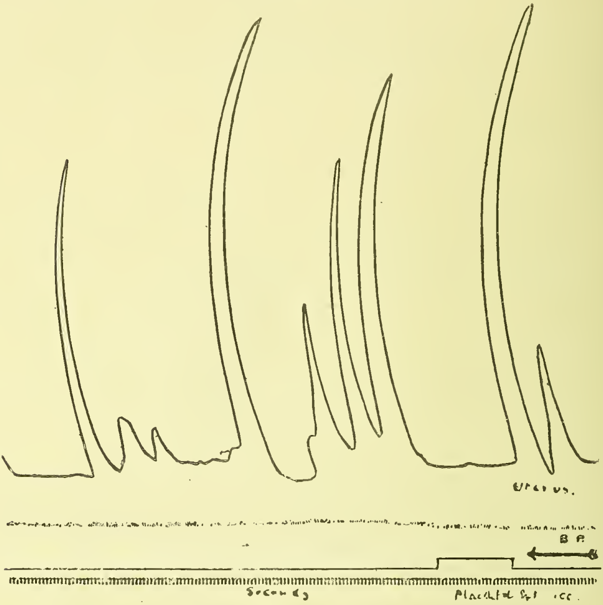

Fig. 14.—Menstruating uterus: effect of placental extract (1 c.cm.). (Reduced by one-half.)

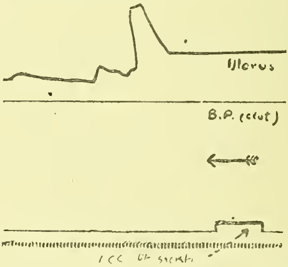

Fig. 15.—Inactive uterus: effect of uterine secretion. (Reduced about one-half.)

(*a*) In Fig. 15 is seen the result upon the inactive uterus. There is an immediate and marked contraction, followed by several smaller ones.

(*b*) Upon the menstruating uterus the effect appears to be that the contractions become prolonged considerably, and occur more frequently. In Fig. 16 this is well seen, but apparently there is a slight delay in bringing about the result. This, however, may have been due in this case to fatigue.

(*c*) Upon the pregnant uterus, as one would expect, we obtained a very powerful reaction. The uterus was thrown into a series of violent and continuous contractions. This is seen in Fig. 17. There were no general convulsions, so that the effect appears to be specific.

Uterine secretion appears to raise the blood pressure, but owing to the marked rapidity with which clotting occurred in the cannula no very good tracings of the blood pressure were obtained. There was, however, no general intravascular clotting.

7. *Calcium Salts.*

One of us (W. B. B.) having already observed an increase in the calcium content of the systemic blood of pregnant women towards the termination of the intrauterine life of the fetus, and taking into consideration the important part played by the calcium salts in all processes associated with female genital activity (see previous papers), we thought that our work on uterine contractions would not be complete unless we endeavoured to show the effect produced upon these contractions by such an important normal constituent of the blood.

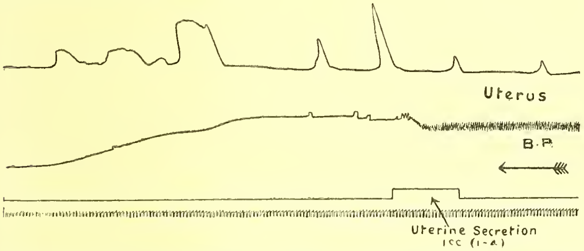

Fig. 16.—Menstruating uterus: effect of uterine secretion. Note clotting in the blood-pressure cannula. (Reduced about one-half.)

For the purpose of intravenous injection we found that a solution of 0.1 gram in 1 c.cm. of calcium chloride was the best suited to our purpose.

(*a*) We were unable to produce any effect upon the inactive uterus.

(*b*) Upon the menstruating uterus a marked effect was produced, as is seen in Fig. 18. From a somewhat weak and prolonged contraction the character was changed so that the expulsive contraction became more powerful and definite, without much loss in the length of the total contraction. We noticed, however, that when the uterus was tired the contraction was apt to be of a more prolonged and tetanic character in addition to being increased in force, and further that the effect was somewhat delayed after the injection. (See Fig. 19.) This delay we fre-

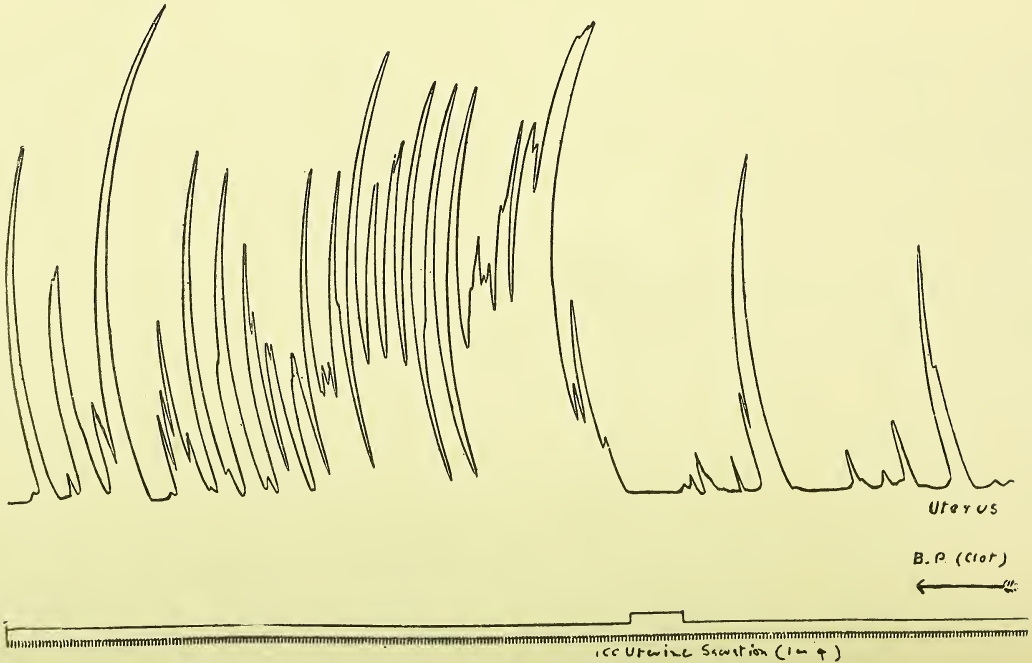

Fig. 17.—Pregnant uterus: effect of uterine secretion. (Reduced about one-half.)

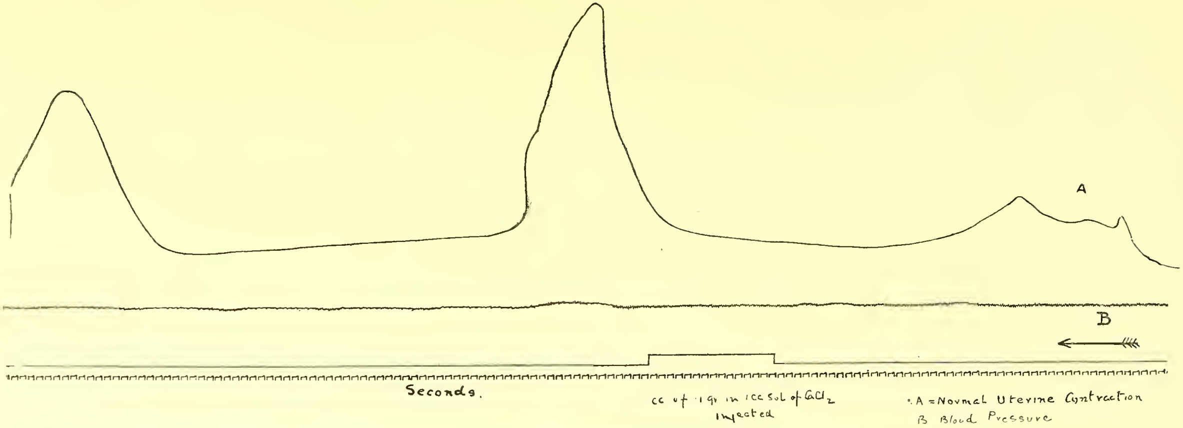

Fig. 18.—Menstruating uterus: effect of calcium chloride (0.1 gram in 1 c.cm.). (Reduced about one-half.)

quently noticed when trying the effect of calcium salts upon the uterus.

(*c*) Upon the pregnant uterus the effect is, as we should expect from our previous observations, very well marked. This is seen in Fig. 20. In some of the tracings obtained the effect was more violent than in the case illustrated.

Calcium salts cause the blood pressure to rise. This, though not nearly so marked as with pituitary and adrenal extracts, is often quite distinct, as is well shown in Fig. 20.

As we have said elsewhere, we have not found that injections of calcium salts cause intravenous clotting.

REMARKS AND CONCLUSIONS.

Such, then, are the results of the investigations which have occupied much of our time and exercised much of our patience. On account of limitations in the former we have not been able to carry out other experiments on the same lines as we had hoped. Some day we may be able to return to the subject; if so, we shall try the effect of extracts made from the whole uterus in order to see if a marketable extract can be made which will have the same definite effects upon uterine contractions as the uterine secretion has without, possibly, the marked effect of the latter upon the coagulation of the blood, an effect possibly produced by nucleo-proteids.

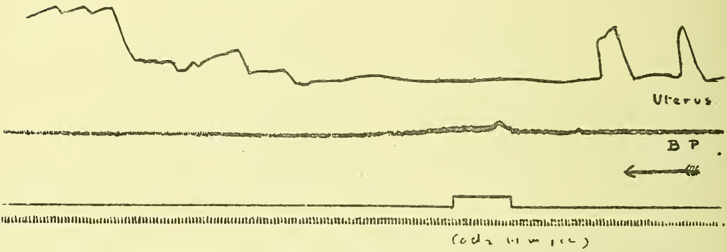

Fig. 19.—Menstruating uterus: delayed effect of calcium chloride. (Reduced about one-half.)

We should like to test the action of fresh blood from a parturient rabbit upon one less advanced in pregnancy. Also, if possible, to have discovered some substance circulating in the blood which definitely inhibits uterine contractions.

Our results, then, indicate that all those substances—most of them being extracts prepared from the ductless glands—which we have investigated appear to increase uterine contractions, and that most raise the blood pressure, some much more than others.

We do not know whether it is quite logical to come to such a conclusion, but it seems to us that probably those extracts such as the pituitary and adrenal—we will omit placental and uterine extracts for the moment—which cause contractions in the inactive uterus act not specifically upon the uterine muscle, but upon involuntary muscle in general. In regard to these, their marked effect upon the blood pressure gives further proof of this.

Of the others, thyroid extract lowers the blood pressure but increases the uterine contractions. In this case, however, the contractions may form part of a general convulsion. Should this occur the blood pressure rises. We think, therefore, that thyroid extract may be dismissed from the competition for specific effect.

Next we have to consider the claims of ovarian extract; and we must confess at once that we wish we had been able to extend considerably our observations in this direction, especially in the matter of using more than one preparation of this organ. We should have liked to note the comparative effect of the extract prepared from rabbits' ovaries during the inactive, menstruating, pregnant, and lactating periods, for we should not be surprised to find different results would be obtained with preparations obtained in the different circumstances. That result in which delay in the contraction of the pregnant uterus occurred is full of significance, and it may be that the ovary of pregnant animals controls uterine contractions, especially in their expulsive effects.

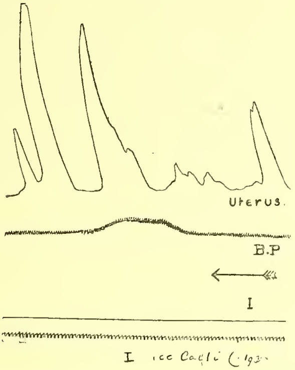

Fig. 20.—Pregnant uterus: effect of calcium chloride. (Reduced about one-half.)

In regard to the secretion obtained from the uterus we were much interested. That it has an effect upon the inactive uterus is undoubted, so that one might be tempted to class it with pituitary, adrenal, and placental extracts. It does not, however, raise the blood pressure in the way that the two first do.

The uterine secretion and placental extract are somewhat similar, then, in their action, and possibly may contain the same substance which effects uterine contractions. We are informed, however, that the specificity of placental extract has been disproved, and that its effects are produced by the products of decomposition it has been found to contain. We have ourselves no first-hand knowledge upon this point.*

Uterine secretion, we venture to think, however, must be specific to some extent, as we have used it straight from the living rabbit to the one under experimentation. Indeed, it seems only feasible that it should have some such action, and the fact that during menstruation, when this substance is being excreted, marked contractions are constantly in progress makes it a fair assumption that these contractions are caused by the direct stimulation of the compound substance we may call " uterin " which is in process of excretion, and which may in some form exist in the blood.

Finally the calcium salts are shown to exert a definite influence upon uterine contractions, with a slight but definite rise of blood pressure. This action is well illustrated, both upon the heart and upon the uterus. A slow, forcible, and rhythmical contraction is produced—the very type that is seen in labour.

We admit that we have been unable in our experiments so far to show definitely that labour *is* produced by the action of calcium salts in the blood, but we have gone some way towards this, especially in that we have previously shown (see Paper II) that uterine secretion is rich in calcium salts. Consequently of the various substances used uterine secretion and calcium salts appeared to us to possess most points in favour of specific action.

This question of specificity in the matter of uterine contractions is, then, at once of considerable difficulty and of great importance. We do not think that until the further investigations indicated have been carried out it can be authoritatively stated that one reagent alone is responsible therefor—for the contractions in menstruation and labour.

So far as we have gone we incline to the belief that calcium salts circulating in the blood, or excreted by the uterus, play the most important rôle in this connexion. At the same time we are alive to the probability that they may act in conjunction with other substances elaborated by such glands as the infundibular portion of the pituitary, either in a hormonic or conjunctive manner. We feel assured, however, that the secret is not now deeply hidden in view of the observations already made.

Contractions of the Human Uterus.

From our observations upon the uteri of rabbits, we may assume that the contractions of the human uterus are governed by the following circumstances:

First, that the normal non-menstruating, non-pregnant uterus does not contract at all. Secondly, that the menstruating uterus is in a state of constantly recurring expulsive contractions. And thirdly, that the normal pregnant uterus contracts and relaxes in a complementary fashion, no expulsive force being exercised; but that during labour violent expulsive contractions occur.

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*Since writing this we have been favoured with a letter from Dr. O. Rosenheim in which he confirms the above statement. This disposes of the claim put forward by Professor Dixon and Dr. F. E. Taylor in regard to the specific action of placental extract.

Therapeutics.

In regard to any therapeutic suggestions we might make we feel that the subject is too big and too uncertain to do justice to here, so that we will content ourselves with one or two remarks only. Until a satisfactory uterine extract is upon the market or the specific (?) calcium salt synthetized, we think that we have a very efficient therapeutic product in the pituitary extract, which we have reason to believe Messrs. Burroughs and Wellcome will shortly put upon the market in hypodermic form. We have ourselves used this preparation clinically with satisfactory results. A dose of 2 c.cm. of the infundibular extract (0.1 gram. in 1 c.cm.) should be given hypodermically and repeated if necessary. In addition to the effect upon the uterus it is most valuable in cases of shock in which the blood pressure must be raised, the protracted period during which the pressure is kept up adding considerably to its value.

We also think that it might be used in the place of physostigmine in paralytic distension of the bowel, its action upon the intestinal muscle being well shown in Fig. 20.

Lastly, we would call the attention of physicians to the effect of the calcium salts upon the heart as shown in the series of tracings made by us for Sir James Barr's paper on Mitral Stenosis (see BRITISH MEDICAL JOURNAL, December 19th, 1908, p. 1797). It appears to us that where it is necessary to slow the heart and strengthen the heart beat, calcium salts administered hypodermically or intravenously should form an ideal drug. In further connexion with this point our remarks on exophthalmic goître in Paper I may be referred to.

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While much remains to be learnt in regard to many of the problems upon which we have endeavoured to throw some light, we hope that the results we have already obtained, and summarized in these four papers, will not only justify the time, thought, and labour expended upon them, but also induce others to complete and extend the work we have so imperfectly performed in the much-neglected branch of gynaecological physiology.

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